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OPTICAL PROPERTIES OF AMORPHOUS AND A1-TYPE ANTIMONY TELLURIUM AND IODINE

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Measurements were made of reflectance, transmittance and coating thicknesses of optical systems consisting of glass substrates coated at 4.2°K with amorphous Sb, Te or I single layers or with B1-type CaO base films and A1-type Sb, Te or I pseudomorphic overlays. A combination of the measured values allowed us to determine the optical constants and the reflectance of amorphous and A1-type Sb, Te and I. The results obtained in the 0.6–4 μ spectral interval (corresponding to the 2.07–0.31 eV photon energy range) for films thicker than 70 nm (where size effects were found to vanish) are presented and discussed here. Let us emphasize that the amorphous phase exhibits semimetallic (Sb) or semiconducting (Te and I) behaviour, whereas the A1-type modification behaves in typically metallic fashion.

INTRODUCTION

A1-type metallic superconducting Sb [1,2], Te [3,4] and I [5] were obtained in the form of metastable pseudomorphic overlays by vapour-quenching at 4.2°K on very smooth, optically polished alkali-zinc borosilicate glass substrates that had been quench-coated at the same temperature with base films of B1-type oxides able to promote pseudomorphism: MnO or CaO for Sb and Te; CaO or SrO for I. The aim of this paper is to present and discuss the findings of a study of the optical constants, in the 0.6–4 μ wavelength range, of the A1-type modification quench-condensed on CaO, which are compared with those of an amorphous Sb, Te and I phase that forms when the quench-deposition takes place in the absence of the base film. The study has been completed by using the optical constants of the A1-type and amorphous modifications for calculating their reflectance at normal incidence.

EXPERIMENTAL

The specimen preparation technique was detailed elsewhere [1,3,5], together with a description of the base-film and overlay crystal structure and the overlay electrical properties in the normal and superconducting states. It must be pointed out that the films were deposited at 0.8–1.2 nm/sec rates in a vacuum chamber which operated at about 10^{-9} Torr and that the residual gas was rarefied air. To determine the optical constants, combined measurements were made of the following quantities: the reflectance at the film-coated substrate side, R, of the substrate/base-film and substrate/base-film/overlay systems or the substrate/amorphous-film system; the transmittance T of the substrate/base-film/overlay system or the substrate/amorphous-film system; the thicknesses s_1 and s_2 of the overlay or the amorphous film and the base film. R and T were measured at normal incidence with a double-beam spectrophotometer, precalibrated so that the substrate can be assumed to be semi-infinite. s_1 and s_2 were determined with a quartz-crystal oscillator whose resonance frequency changes with mass loading, by deriving the density of the B1-type and A1-type films from the lattice constant deduced from electron-diffraction patterns and assuming that the density of the amorphous films is the same as that of the ordinary A7-type Sb, A8-type Te and orthorhombic I (which is quite reasonable, since careful electron-diffraction and electron-microscope investigations show that in effect the amorphous films have an amorphous-like microcrystalline structure and consist of very fine grains of the above types). The study was restricted to specimens with s_1 = 10–100 nm and $s_2 > 10$ nm, since deposits thinner than 6–8 nm are not yet isotropic and homogeneous, as shown by electron-transmission micrographs, and A1-type overlays thicker than 110–120 nm are already too absorbing for a reliable measurement of T.

THEORETICAL CONSIDERATIONS

R and T of the substrate/base-film/overlay and substrate/amorphous-film systems can be expressed, respectively, by the relations [6,7]

$$R=\left|\frac{r_1+r_2\exp(-2i\alpha_1)+r_3\exp\left[-2i(\alpha_1+\alpha_2)\right]+r_1r_2r_3\exp(-2i\alpha_2)}{1+r_1r_2\exp(-2i\alpha_1)+r_1r_3\exp\left[-2i(\alpha_1+\alpha_2)\right]+r_2r_3\exp(-2i\alpha_2)}\right|^2$$

$$T=\frac{n_s}{n_o}\left|\frac{t_1t_2t_3\exp\left[-i(\alpha_1+\alpha_2)\right]}{1+r_1r_2\exp(-2i\alpha_1)+r_1r_3\exp\left[-2i(\alpha_1+\alpha_2)\right]+r_2r_3\exp(-2i\alpha_2)}\right|^2 \qquad (1)$$

$$R=\left|\frac{r_1+r_2\exp(-2i\alpha_1)}{1+r_1r_2\exp(-2i\alpha_1)}\right|^2,\quad T=\frac{n_s}{n_o}\left|\frac{t_1t_2\exp(-i\alpha_1)}{1+r_1r_2\exp(-2i\alpha_1)}\right|^2, \qquad (2)$$

where n_s and n_o are the indices of refraction of the substrate and vacuum, r_i and t_i are the amplitude reflection and transmission coefficients at the overlay/vacuum or amorphous-film/vacuum (i = 1), base-film/overlay or substrate/amorphous-film (i = 2) and substrate/base-film (i = 3) interfaces and α_i is the phase thickness of the overlay or amorphous film (i = 1) and base film (i = 2).

These parameters may be written in the known forms

$$r_1=\frac{n_o-n_1+ik_1}{n_o+n_1-ik_1},\quad r_2=\frac{n_1-ik_1-n_2}{n_1-ik_1+n_2},\quad r_3=\frac{n_2-n_s}{n_2+n_s}$$

$$t_1=\frac{2n_o}{n_o+n_1-ik_1},\quad t_2=\frac{2(n_1-ik_1)}{n_1-ik_1+n_2},\quad t_3=\frac{2n_2}{n_2+n_s}$$

$$\alpha_1=\frac{2\pi(n_1-ik_1)s_1}{\lambda},\quad \alpha_2=\frac{2\pi n_2 s_2}{\lambda} \qquad (3)$$

where λ is the radiation wavelength, n_1 and k_1 are the optical constants of the overlay or amorphous film (i.e. the real and imaginary parts of its refractive index) and n_2 is the index of refraction of the base film. Note that, obviously, in the coefficients r_2 and t_2 of the substrate/amorphous-film system n_2 must be replaced by n_s and that n_2 can be derived from the measured values of s_2 and reflectance R of the substrate/base-film system by using the expression [6]:

$$R=\frac{(n_s^2+n_2^2)(n_2^2+n_o^2)-4n_sn_2^2n_o+(n_s^2-n_2^2)(n_2^2-n_o^2)\cos(4\pi n_2s_2/\lambda)}{(n_s^2+n_2^2)(n_2^2+n_o^2)+4n_sn_2^2n_o+(n_s^2-n_2^2)(n_2^2-n_o^2)\cos(4\pi n_2s_2/\lambda)} \qquad (4)$$

Introducing eqs. (3) into (1) and (2), and inserting the measured values of R, T, s_1 and s_2 and the values of n_2 deduced from (4), a system is obtained whose unknowns are n_1 and k_1. Eq. (4) and this system were solved by limited series expansions of the transcendental functions. The results found for $s_1 > 70$ nm (where n_1 and k_1 exhibit size-independent bulk values n_{1b} and k_{1b}) in the spectral interval from 0.6 to 4 μ (corresponding to the 2.07–0.31 eV photon energy range) are shown in Figs. 1 – 3 together

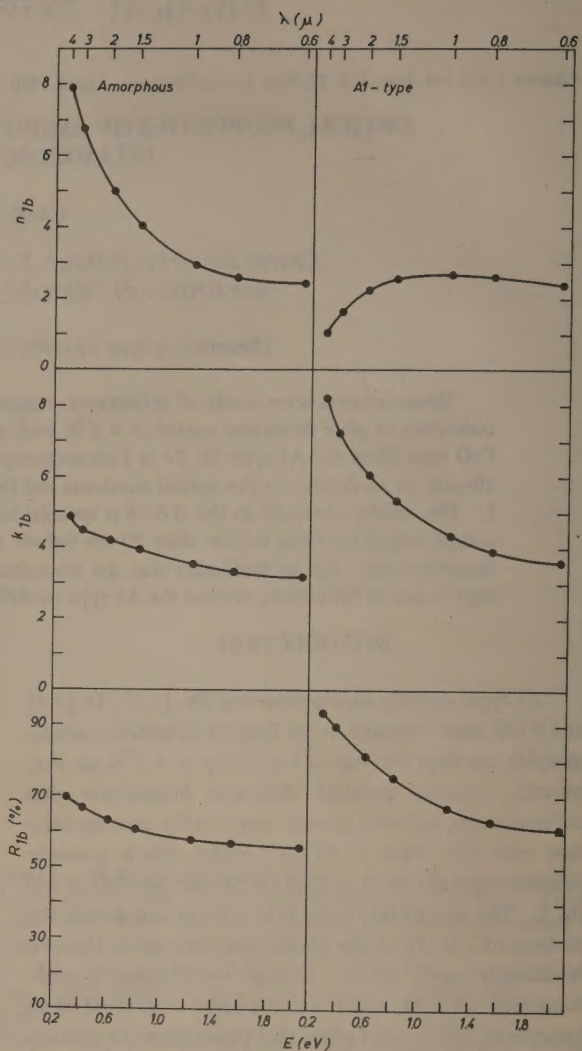

Fig. 1. Photon energy and wavelength dependence of the optical constants n_{1b} and k_{1b} and the reflectance at normal incidence, R_{1b}, of amorphous and A1-type bulk Sb. The circles represent values determined by averaging those derived from measurements of reflectance R, transmittance T and coating thicknesses of a large number of glass plates coated with amorphous Sb or B1-type CaO and A1-type Sb. R, T and the thicknesses were measured with a precision of about ± 0.1%. This accuracy, together with the observed departure of the values obtained for some specimens from an ideal Gaussian-type distribution, results in a maximum error of ±1.2% (not shown in the figure) in the determination of n_{1b} and k_{1b}.

with the values of the bulk reflectance at normal incidence of the A1-type and amorphous phases, derived from the well-known formula

$$R_{1b}=\frac{(n_{1b}-n_o)^2+k_{1b}^2}{(n_{1b}+n_o)^2+k_{1b}^2} \qquad (5)$$

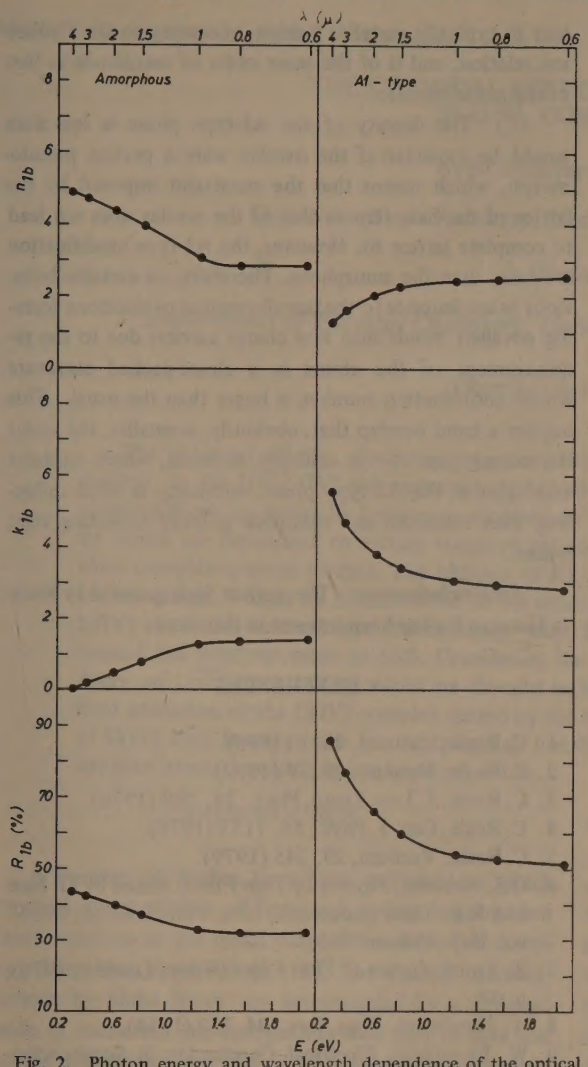

Fig. 2. Photon energy and wavelength dependence of the optical constants n_{1b} and k_{1b} and the reflectance at normal incidence, R_{1b}, of amorphous and A1-type bulk Te.

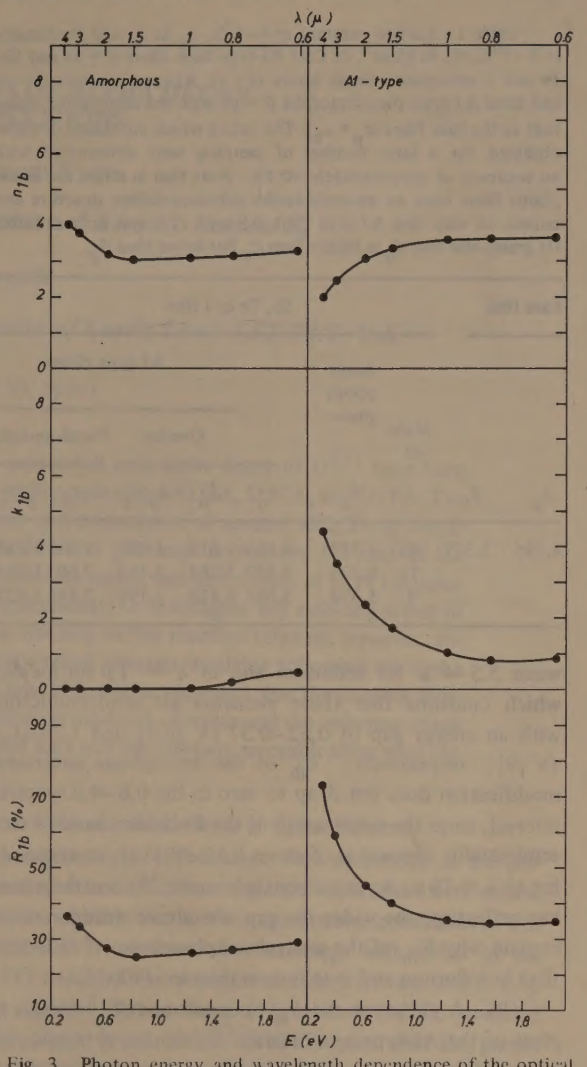

Fig. 3. Photon energy and wavelength dependence of the optical constants n_{1b} and k_{1b} and the reflectance at normal incidence, R_{1b}, of amorphous and A1-type bulk I.

The structural data are given in Table 1.

RESULTS

The findings of the present investigation may be summarized as follows:

Real Part of the Refractive Index. n_{1b} is higher in the amorphous than in the A1-type material, except for antimony at λ =0.6–0.8 μ and iodine at μ =0.6–0.2 μ. n_{1b} of the amorphous phase increases with λ, except for iodine at λ =0.6–1.5 μ, whereas n_{1b} of the A1-type modification behaves oppositely, except for antimony at λ =0.6–1 μ.

Imaginary Part of the Refractive Index. k_{1b} is always higher in the A1-type than in the amorphous material. k_{1b} of the amorphous phase increases with λ in antimony and decreases with increasing λ in tellurium and iodine, whereas k_{1b} of the A1-type modification increases with λ in all three materials, except for iodine at λ = 0.6–0.8 μ.

Reflectance. R_{1b} is always higher in the A1-type than in the amorphous material. R_{1b} increases with λ in both amorphous and A1-type materials (except for the amorphous tellurium at λ = 0.6–0.8 μ, amorphous iodine at λ = 0.6–1.5 μ and A1-type iodine at λ = 0.6–0.8 μ), but the increase is much sharper in the A1-type than in the amorphous modification.

DISCUSSION AND CONCLUSIONS

(A) k_{1b} of the amorphous phase drops to zero at λ bet-

Table 1. Lattice constant at 4–7°K, a_i, in Å, and /or density at 4–7°K, d_i, in g/cm^3, of CaO B1-type base films (i = b) and Sb, Te and I amorphous single layers (i = a), A1-type overlays (i = o) and ideal A1-type pseudomorphs (i = p) with the same lattice constant as the base films ($a_p = a_b$). The values which are means of those obtained for a large number of samples, were determined with an accuracy of approximately +0.1%. Note that in effect the amorphous films have an amorphous-like microcrystalline structure and consist of very fine A7-type (Sb), A8-type (Te) and orthorhombic (I) grains and that d_o is higher than d_a but lower than d_p.

Base film		Sb, Te or I film							
				A1-type phase					
		Material	Amorphous phase	Overlay			Pseudomorph		
a_b	d_b		d_a	a_o	d_o	d_o/d_a	d_p	d_p/d_a	
4.795	3.379	Sb	6.742	4.866	7.019	1.041	7.335	1.088	
		Te	6.325	4.887	7.261	1.148	7.687	1.215	
		I	5.374	5.081	6.428	1.196	7.648	1.423	

ween 3.5–4 μ for tellurium and at $\lambda \approx 1\,\mu$ for iodine, which confirms that these elements are semiconductors with an energy gap of 0.32–0.37 eV [8,9] and 1.24–1.3 eV [9], respectively; k_{lb} of the amorphous antimony modification does not drop to zero in the 0.6–4 μ spectral interval, since the mean width of the forbidden band of this semimetallic element is of about 0.12 eV [10], corresponding to $\lambda \approx 10\,\mu$. As an element is less metallic, and therefore less reflecting, the wider the gap, the above considerations explain why R_{lb} of the amorphous phase is lower in iodine than in tellurium and in tellurium than in antimony.

(B) A glance at the k_{lb} versus λ and R_{lb} versus λ plots of the A1-type modification shows that it behaves as expected for typical metals, which have no energy gap. This is consistent with the results of previous investigations, which showed that the A1-type antimony [1], tellurium [3] and iodine [5] may become superconductors and that in the normal state their resistivity increases with temperature in typically metallic fashion, according to the Grüneisen relation, and is of the same order of magnitude as that of the noble metals.

(C) The density of the A1-type phase is less than would be expected if the overlay were a perfect pseudomorph, which means that the constraint imposed by the lattice of the base film to that of the overlay does not lead to complete lattice fit. However, the A1-type modification is denser than the amorphous. Therefore, its metallic behaviour is attributable to the transformation of electrons forming covalent bonds into free charge carriers due to the rearrangement of the atoms in a closer-packed structure whose coordination number is larger than the usual. This implies a band overlap that, obviously, is smaller, the wider the energy gap of the ordinary material, which explains why, also in the A1-type phase, antimony is more reflecting than tellurium and tellurium is more reflecting than iodine.

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THE CHEMICAL EFFECTS OF ALPHA DECAY IN U^{235} COMPLEX IONS

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The chemical effects of nuclear transformation associated with alpha decay of U^{235} have been studied in the U^{235} (IV) polyaminopoly carboxylic acid complexes of EDTA, DCTA, and DTPA. The observed break up values lie between a minimum of zero and a maximum of around 40%. These break-up values are dependent on certain ranges of pH and a different pH range is observed for each of the three complex systems studied. The addition of KI, a reducing agent, and an increase of U(IV) concentration (about 7 times its concentration in the other experiments) to investigate any reducing effect of U(IV) ion itself, does not alter the break-up value. The freezing of the reaction mixture, however, enhanced the break-up value to 56%. Considering the high recoil energies available following the alpha decay of U^{235} the break up values are thought to be low. It has been suggested that the possible localised oxidation of the U(IV) complex caused by the radiolysis products of water and the reducing effect of U(IV) ions, is responsible for the release of free ligand ions and the ultimate recombination with the daughter atoms, resulting in low break-up values.

INTRODUCTION

A number of studies have been published in which chemical effect studies of beta and gamma type nuclear transformations in the metal polyaminocarboxylate complexes have been described [1–11]. The nuclear transformations by alpha decay are accompanied by a different mode of excitation and energisation than that of beta and gamma transitions and they have not been extensively studied. Whereas, in the case of beta and gamma decays recoil energies being low, bond rupture has been attributed to the production of multiple charged states of the daughter atoms [1–11] (formed by processes like Auger effect, internal conversion and shake-off), the alpha decay effects may be considered to be result of primarily the recoil energy imparted to the daughter atom following alpha emission. In this work studies have been carried out to determine the role of these bond rupturing origins in the alpha decay of U^{235} which is complexed with the multidentate ligands; EDTA, DCTA and DTPA. Both uranium and its daughter thorium, being in the actinide family, have analogous chemical properties and they differ only by two in atomic number. Also uranium in its (IV) oxidation state and its duaghter Th(IV) form very stable complexes [12, 13] with EDTA, DCTA and DTPA. Therefore it was possible to set up favourable conditions for the investigation of the chemical effects of alpha emission.

Alpha decay experiments were performed in the presence of free Cd^{2+} ions. Separate experiment were carried out to establish that in the experimental conditions of this study Cd^{2+} ions have slow exchange with either of the U(IV) and Th(IV) complexes and they are also able to stop the reported fast exchange [14] of free Th(IV), produced after alpha decay of U^{235}(IV) complex with the parent complexed ion.

EXPERIMENTAL

Reagents. Cd^{2+} stock solutions were prepared by the dissolution of accurately weighed amount of $CdCl_2$ (Koch Lab) in approximately $10^{-4}M$ HCl. The A. R. disodium salt of EDTA was dissolved in HCl and then neutralized by ammonia to precipitate the pure anhydrous H_4EDTA solid, whereas pure H_4DCTA and H_5DTPA were obtained by twice recrystallizing the commercial samples of the acids from hot water. U^{238} stock solutions were prepared by dissolving A.R. uranyl nitrate (B.D.H.) in 0.2*M* HCl, these solutions were then subjected to catalytic hydrogenation [15] in order to prepare U^{238}(IV) stock solutions. U^{235} tracer solutions in 3*M* HNO_3 were obtained from Radiochemical Centre, Amersham, England. HNO_3 was removed

Table 1. Radiochemical composition of the U^{235} solution.

Isotope	U^{235}	U^{234}	U^{236}	U^{238}
Percentage	93	0.75	0.25	6
Total alpha activity in nano curies in 200 mg sample	428	9130	29.78	3.97

by evaporation and the residue was redissolved in 0.2*M* hydrochloric acid. The solution was found to have traces of copper which interfered in catalytic hydrogenation of U(VI) to U(IV) and, therefore, these traces of copper were removed by an anion exchange separation [16] procedure prior to reduction. Another complication was experienced due to the radiochemical composition of the U^{235} sample. The sample contained high specific activity alpha emitting isotopes like U^{234} and U^{236}. Table 1 shows the distribution of the various isotopes of uranium in the Amersham U^{235} samples found by mass spectrometric analysis. The presence of these other alpha emitters complicated the radiochemical assay of the U^{235} atom, and hence that of the total daughter atoms, produced with in the decay period.

Cation Exchange Resin. Zeo-carb 225 cation exchange resin less than 200 mesh was used in the sodium form.

Analytical Techniques. U(IV) stock solutions were analysed by three methods to give identical results. They were: (1) potentiometric titrations [17] with standard solutions of $K_2Cr_2O_7$, (2) volumetric [17] titrations with standard $K_2Cr_2O_7$, and (3) the complexometric titrations [18] with standard EDTA solutions, whereas the solutions of uranous complexes of EDTA, DCTA and DTPA were analysed by volumetric titrations against standard ceric sulphate solutions [19]. The analysis of the carbon, hydrogen and nitrogen content of the complexes was carried out by the departmental microanalyst. The results were accurate within a limit of ± 0.3%.

Measurement of Radioactivity. All the samples were counted on the Packard 3310 Tricarb liquid scintillation counter. The two ml aqueous solution, to be counted; was mixed with 10 ml organic scintillation solution, prepared according to the fomula of Bruno and Christian [20], without the occurrence of any phase separation. The chemical composition of each of the samples was kept constant in order to ensure the same degree of quenching. The discriminator levels of the instrument were set in such a way that the 4–5 meV alpha particle pulses of uranium isotopes were excluded and only 0.3 meV Th^{231} beta energy pulses were recorded; oxalic acid which was used to elute the Th^{231} free ions from the cation exchange column was found to quench the liquid scintillation counting of Th^{231}. The extent of this quenching was experimentally determined and a proper allowance was made for it when oxalic acid containing activity was compared with the activi of the samples, not containing oxalate ions.

Procedure

Both the active and inactive U(IV) Y complexes were prepared by normal procedure [21]. The U(IV) content of both complexes was determined by analytical methods described earlier. C, H and N analysis of the active U^{235}(IV) complexes was not practicable, however U^{238}(IV) complexes were precipitated in identical condition and they were microanalysed. These precipitated complexes were dissolved in appropriate HCl solutions to give the required stock solutions at known pH.

The reaction mixtures were prepared in 100-ml volumetric flasks, sealed with serum caps to avoid oxidation. All the solutions were deoxygenated by bubbling with white spot nitrogen. The reaction mixtures contained the following:

Complex solutions of U^{235} (IV), 25–30 ml, at an appropriate pH, are maintained by varying amounts of acid concentration of the solution. It also contained one ml of the standard $CdCl_2$ solution which was introduced in the reaction mixture to initiate the reaction. Before the addition of Cd^{++} free ions all of the U^{235} (IV) and Th^{231} (IV) ions will be in the complex form. A decay period of seven days was given to ensure that almost all of the Th^{231} (t½ 25.6 hr) already present had decayed out and fresh Th^{231} with measurable activity was grown in.

Total Activity Measurement. Two ml of the reaction mixture was counted on the Packard 3310 liquid scintillation counter within a limited discriminator level setting of A/B = 50/350 at an optimum gain of 11%. In a separate experiment it was ensured that this range of window opening of the instrument excludes essentially all of the alpha particles pulses from uranium isotopes. The activity recorded by the sample was taken to be the total activity, i.e. the total Th^{231} produced in the reaction mixture in any form, either complexed or uncomplexed.

Free Ion Activity Measurements. Within a period of seven days a measureable amount of Th^{231} activity would be expected to be accumulated in the free ion form, owing to the molecular disruption of the Th^{231} complexes of EDTA, DCTA and DTPA which are produced as a consequence of alpha decay of the corresponding U^{235} complexes. Therefore, a 5-ml aliquot of the reaction mixture

was taken out and forced through cation exchange column by nitrogen pressure, to absorb any Th^{231} free ions present in the reaction mixture. The cation exchange column was washed with deionized water and the effluent alongwith the washings was collected in a 25-ml volumetric flask (will be called effluent hereafter). The Th^{231} ions, absorbed on the column were then eluted with 2 ml 1*M* oxalic acid solution. Then the column was washed three times with a 1-ml portion of deionized water. The eluent and the washings were collected in a 5-ml volumetric flask (called eluent hereafter). Separate experiments had shown that 99.9% of the thorium was eluted by this method. Two ml of the eluent was then counted in the Packard scintillation counter in exactly the same way as the total activity sample. Free ion Th^{231} containing samples (eluent samples) were counted about every 400 min for the first three to four days and then at least twice a day for a total period of 10 days in order to obtain a precise decay curve of Th^{231} atoms. ($t½$ 25.6 hr).

Effluent Activity Measurements. In the reaction mixture Th^{231} is always in radiochemical equilibrium with the parent U^{235} activity. When the mixture is passed through the ion exchange column it loses its Th^{231} free ions and hence the radioactive equilibrium is disturbed in the complex ion fraction of the reaction mixture which we get in the form of effluent. In order to reestablish the equilibrium, Th^{231} activity will be expected to grow in the effluent. Therefore, the growth of Th^{231} activity in the effluent was also followed by counting an aliquot of the complex ion fraction of the reaction mixture under the same conditions in which other two samples were counted. Growth of Th^{231} atoms in the effluent was followed until a constant value was obtained.

Check for the Extent of Oxidation in the Radioactive Mixture. Oxidation of the U(IV) complex in the reaction mixture was expected to yield free uranyl ions [21]. Therefore, a 2-ml aliquot of reaction mixture was passed through a cation exchange column in order to ensure the removal of the free uranyl cations from the reaction mixture. Total activity of the reaction mixture, the activity recorded by counting the sample in the Packard counter with a fully open window of 50 to infinity, at 11% gain, so that all the alpha particles of uranium could be counted, was compared with the total activity of the complex ion fraction, i.e. effluent, counted in the identical conditions. In those cases where there was not any difference in the alpha count rates of the reaction mixture and the effluent, it was assumed that oxidation has not taken place.

Checks for Precipitation and Adsorption. Two equal aliquots from the reaction mixture, at the end of each run were counted with window settings of 50 to infinity and it was found that identical counts were registered for each sample and hence precipitation and adsorption were shown to be absent.

RESULTS

Free Cd^{2+} ions were introduced to the reactions mixtures, prepared for the study of alpha decay in U^{235} chelate complexes. These were added to scavange the free ligands, generated by the expected bond rupture of the daughter Th^{231} complex ions produced as a consequence of alpha decay of corresponding U^{235} chelate complexes. In a separate set of experiments it was established that under these experimental conditions: (1) free Cd^{2+} ions have a slow rate of exchange with the U(IV) and Th(IV) complexes of EDTA, DCTA and DTPA and, therefore, undesirable redistribution of activities in the free ion and complexed ion form is not possible; (2) these free ions are able to stop the possible exchange [14] of free Th(IV) ion (tracer amount), produced after alpha decay of the U^{235}(IV) complexes, with the parent complex, as free Th(IV) ions were found to stick on cation exchange column when solutions of identical composition were passed through them. The reactions which proceed in the reaction mixture are considered to be the following:

$$M_pL \xrightarrow{\text{Alpha decay}} M_d + L$$

$$L + M_s \longrightarrow M_sL$$

Where M_p, M_d and M_s stand for the parent, daughter and the scavanger metal ions respectively, and L, the free ligand. Therefore, the percentage bond break up was determined by the equation:

$$X = \frac{N_d^f}{N_d^T} \times 100$$

Where N_d^f is the number of daughter atoms observed in the free ions form as a consequence of molecular disruption because of alpha decay and N_d^t is the total number of such atoms generated (either free or bound, within the decay period).

The percentage break-up observed under the various conditions such as, variation of pH, ligand and freezing of the reaction mixture are shown in Tables 2 – 4 and Fig. 1 depicts the pH dependence of the 'X' values. In order to examine the likely effects, of the presence of a reducing agent on the percentage break up results, two separate experiments were carried out. Two reducing agents em-

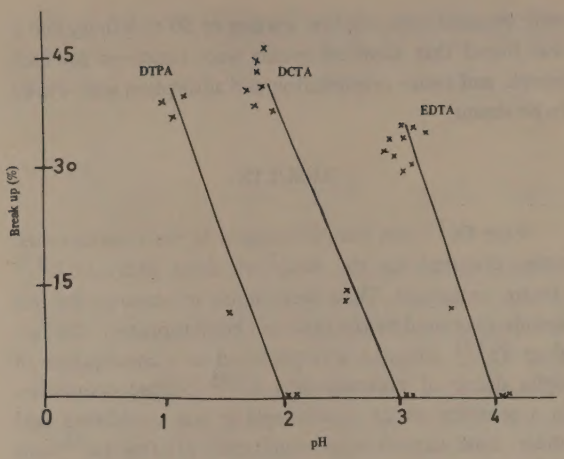

Fig. 1. Results of $U^{235} \xrightarrow{\alpha} Th^{231}$ experiments.

Table 2. Percentage break-up results for the U^{235}(IV) – EDTA system at 21°

U^{235}(IV) EDTA (*M*)	Cd^{2+} (*M*)	pH ± 0.001	Break-up percentage ± 5%
6.60×10^{-4}	1.6×10^{-5}	3.234	33.1
8.81×10^{-4}	"	3.212	33.0
9.42×10^{-4}	"	2.988	33.9
1.93×10^{-3}	3.2×10^{-5}	2.899	32.1
2.08×10^{-3}	3.6×10^{-5}	2.978	32.5
2.18×10^{-3}	3.2×10^{-5}	3.123	34.9
1.30×10^{-3}	"	3.124	33.1
1.84×10^{-3}	"	4.236	0
2.28×10^{-3}	"	2.125	0
1.18×10^{-3}	1.6×10^{-5}	3.189	29.8
1.18×10^{-3}	"	3.189	30.2
8.80×10^{-3}	"	2.989	33.0
1.30×10^{-3}	"	3.725	8.0

ployed for this study were KI and U(IV) itself, thought to have more powerful reducing effect as compared to $Na_2S_2O_3$ used by Asano [11]. The results of the per cent break-up observed in the presence of $10^{-2}M$ KI and in the presence of 7 times excess U(IV) in the form of U^{238}(IV) DTPA are shown in Tables 6 and 7 which are not different from the earlier results. However, the freezing of the reaction mixture of U(IV) DTPA system at pH 1 enhanced the percentage break-up value as shown in the Table 5.

DISCUSSION

From these results the following points would appear

Table 3. Percentage break-up results for the U^{235}(IV) DCTA system at 21°

U^{235}(IV) DCTA (*M*)	Cd^{2+} (*M*)	pH ± 0.001	Break-up percentage ± 5%
1.26×10^{-3}	3.2×10^{-5}	3.188	0
6.05×10^{-4}	1.6×10^{-5}	1.889	41.3
6.40×10^{-4}	"	1.983	42.7
6.40×10^{-4}	"	1.994	41.7
2.00×10^{-3}	3.2×10^{-5}	2.123	41.9
4.18×10^{-4}	1.60×10^{-5}	1.925	44.1
1.15×10^{-3}	6.4×10^{-5}	2.246	40.0
5.73×10^{-4}	"	2.112	41.7
1.78×10^{-3}	"	2.598	13.7
1.72×10^{-3}	"	1.598	10.9
1.56×10^{-3}	"	1.562	44.5
1.32×10^{-3}	"	3.135	0

Table 4. Percentage break-up results for the U^{235}(IV) – $DTPA^{-}$ system at 21°.

U^{235}(IV) $DTPA^{-}$ (*M*)	Cd^{2+} (*M*)	pH ± 0.001	Break-up percentage ± 5%
8.83×10^{-4}	1.6×10^{-5}	2.307	0
9.63×10^{-4}	3.6×10^{-5}	2.100	0
1.29×10^{-3}	6.6×10^{-5}	2.100	0
1.29×10^{-3}	2.6×10^{-5}	1.667	8.3
1.62×10^{-3}	6.5×10^{-5}	1.193	39.5
9.33×10^{-4}	6.5×10^{-5}	1.009	40.1
2.18×10^{-3}	6.5×10^{-5}	1.012	40.1

Table 5. Percentage break-up results for the U^{235}(IV) – frozen system.

U^{235}(IV) DTPA (*M*)	Cd^{2+} (*M*)	pH	Break-up percentage ± 5%
9.32×10^{-4}	$1.6 \times ^{-1}$		
9.32×10^{-4}	1.6×10^{-5}	1.011	56.6
9.32×10^{-4}	1.6×10^{-5}	1.009	56.6

to emerge: (1) The maximum break-up percentage has been observed to be 40% only. (2) The percentage bond rupture is pH-dependent. (3) A different pH range has been observed for each of the complex system studied. (4) Reducing agents do not seem to affect the break-up result. (5) Freezing of the reaction mixture, produced the bond rupture of higher order. These points are to be

Table 6. Percentage break-up results for the U^{235}(IV) – DTPA system when $10^{-2}M$ KI was added to the reaction mixture.

U^{235}(IV) $DTPA^{-}$ (M)	Cd^{2+} (M)	pH ± 0.001	Break-up percentage ± 5%
1.89×10^{-3}	1.6×10^{-5}	0.890	37.4
1.89×10^{-3}	1.6×10^{-5}	0.910	37.8

Table 7. Percentage break-up results for the U^{235}(IV) – DTPA system when U(IV) concentration was increased in the form of U^{238}(IV) $DTPA^{-}$.

U(IV) $DTPA^{-}$ (M)	Cd^{2+} (M)	pH ± 0.001	Break-up percentage ± 5%
7.88×10^{-3}	3.2×10^{-5}	1.011	42.5
7.88×10^{-3}	3.2×10^{-5}	1.011	42.5

interpreted in view of the following.

The important modes of excitation and ionisation, following alpha decay of U^{235} to Th^{231} are as follows: (1) The production of positive charge on Th^{231} daughter atoms following: (i) shake off-of the electrons, and (ii) internal conversion of the gamma rays. (2) Recoil energies due to the emission of an alpha particle.

Recoil energies, calculated on the basis of the data available in the Nuclear Data Sheets [22] and Table of Isotopes [23] are shown in Table 9 and Table 8 shows the relevant information about gamma rays from the same Nuclear Data Sheet [22]. From Table 9 it is evident that the recoil energies associated with the various alpha particles emitted by U^{235}, are of high values, varying between 73.00 – 79.54 keV. Recoil energies from gamma rays and conversion electrons will be too low to be considered here. The recoil energies can be used to calculate the ranges of alpha decay daughter atoms, by using Bohr's equations [27]. Thus, for the daughter Th^{231} atom recoiling with an energy of 76.12 keV, the range will be 27.1 nm, this distance would be considered to be sufficiently large enough to allow the daughter Th^{231} atoms to escape from the sphere of influence of the fragmented ligand and the initial solvent cage. Hence, every daughter Th^{231} atom produced following alpha decay of U^{235} is expected to break the molecular bonds and come to rest in solution as a free metal ion.

In addition, attention is to be given to the charge production processes in U^{235} alpha decay. The charge spectra of Th^{231} atoms produced as a result of internal conversion of gamma rays following alpha decay of U^{235} has not been reported. However, due to the probability of internal conversion in about 57% of the alpha decay events (Table 8) it is expected that there will be an outburst of conversion electrons from the recoiling Th^{231} daughter atoms in two stages : (i) in 7% of the decay events, when prompt gamma rays are internally converted, the recoil of the daughter atoms and the emission of the electron will be simultaneous, and (ii) the outburst of the second group of electrons, comprising 40% of the decay events, will be in a situation where the daughter atoms have already come to rest.

The low break-up results observed in this work can only be understood, if it is assumed that recombination of the recoil daughter atoms takes place. The interacting ligand can not be its original partner in the complex because our calculations show that the recoil range, is sufficient enough to allow the daughter atom to escape from its initial solvent cage and the sphere of influence of the fragmented ligand.

Table 8. The energies, abundances and total conversion coefficients of the principal gamma-rays found in U^{235} decay.

Gamma-ray energy (meV)	Internal conversion coefficient ($\times 10^2$)	Abundance (%)	Converted (%)	Life time of the excited state
0.201	99.2	3	3.0	
0.180	95.5	2	1.9	
0.204	95.0	5.5	5.2	10^{-15}
0.163	95.0	5.8	5.5	sec
0.109	97.5	2.7	2.1	
0.185	46	60	27.6	0.77×10^{-9}
0.143	89	13	12.6	sec

Table 9. Recoil energies of the Th^{231} daughter atoms.

Alpha particle abundance (%)	Alpha particle energy (meV)	Recoil energy (keV)
4.6	4.597	79.54
3.7	4.556	78.86
1.2	4.502	78.79
4	4.415	76.19
57	4.396	76.12
18	4.366	75.58
3	4.323	74.84
5.7	4.216	73.00

Thus, the cage recombination proposition put forward by Cottin and Haissinsky [24] to explain the low break-up results in their investigation of U^{238} alpha decay in uranyl benzoyl acetonate solution (recoil energy of daughter Th^{234} = 71 keV) must be reconsidered. The recoil ranges lead us to think that the recombination of daughter Th^{231} takes place with a free ligand ion at its place of rest. Let us see how these free ligands are originated. The recoiling Th^{231} atoms will come to rest in a time of the order of 10^{-13} sec and there will be a subsequent outburst of conversion electrons as described earlier. The daughter atoms will be highly ionised and radiolysis of adjacent solvent molecules will occur. The radiolysis of the solvent molecules will also be caused by the dissipation of the last few tens of electron volts of the daughter atoms kinetic energy in the immediate vicinity of its place of rest. A factor of major importance to be considered here is the presence in the reaction mixture of 10^{-3} to 10^{-4} molar concentration of U(IV) complex; U(IV) is a strong reducing agent and may neutralize the ionised daughter atoms and the U(IV) complex ions may additionally react with the radiolysis products of the interaction with the solvent. If either of these processes is carried through, the U(IV) complex ions will be oxidised and form free uranyl and ligand ions. These ligand ions will be then free to react with daughter Th^{231} atoms and thus reduce the amount of break-up which may be observed. In order to explain the pH dependence of the break-up results it can be pointed out that the reducing effect of U(IV) [25–26] increases with the increase of pH, and accordingly pH dominates the break-up values, which decrease with the increase of pH. At the pH where the bond rupture completely disappears, it is likely that the oxidation of U(IV) complex takes place to an extent that it produces enough free ligands for the complete recombination of the free daughter atoms.

The tendency that the daughter complex of higher thermodynamic stability shows break-up in higher acidic concentrations can be explained in terms of the protonation tendency of polyaminopolycarboxylic ligands, which blocks interaction of the ligand with metal ions. Therefore, an increase in the acidity of the solution will decrease the probability of recombination of metal and ligand, and at a particular acid concentration, the probability of the reformation of a complex will be more likely in a complex of higher thermodynamic stability. Thus, we observed complete recombination of Th EDTA (log K = 23.2) at pH 4, of Th DCTA (log K = 24.9) at pH 3 and that of Th DPTA (log K=27) even at pH 2. The addition of KI, a reducing agent, to the reaction mixture, or an increase of U(IV) concentration, approximately 7 times to its normal concentration in the other experiments, in the U^{235} (IV) DTPA system, does not produce any decrease in the break-up percentage, perhaps signifying that the reduction to its limiting value has already been achieved (Tables 6 and 7). However, the break-up percentage increased to about 50% when the U^{235}(IV) DTPA decay was followed in the frozen solutions (Table 5). This might be due to the released electrons from the recoiling atom being trapped in the frozen solution and prevented from producing substantial radiolysis which probably is responsible for the recombination.

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HEATS OF FORMATION OF Zn(II), Al (III) AND B(III) PHOSPHIDES

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The heats of combustion of zinc phosphide, aluminium phosphide and boron phosphide have been determined using oxygen bomb calorimetric techniques. Also the heats of combustion of mixtures of red phosphorus and the corresponding metals (Zn, Al and B), in the stoichiometric ratios as found in each phosphide were determined. From these data, the following heats of formation in kcal/mole, of the respective phosphides have been computed at ca.298°K :

Zinc phosphide, Zn_3P_2, $\Delta H_{298^o} = -90.3 \pm 5.2$; aluminium phosphide, AlP, $\Delta H_{298^o} = -29.0 \pm 1.7$; boron phosphide, BP, $\Delta H_{298^o} = 60.2 \pm 4.6$..

INTRODUCTION

Only a few phosphides have been studied previously with reference to their heats of formation due to difficulties in finding appropriate reactions and the hazards of handling phosphorus and phosphine. Some measurements have been made in this direction for such phosphides as could be prepared easily in the laboratory [1 – 3]. The reported values have been obtained generally by methods [4,5] other than calorimetric. The phosphides of boron and aluminium have been much less investigated in this regard because of the relatively minor importance of these compounds until recently. With the discovery of semiconducting properties of silicon and germanium, considerable interest has generated in the investigation of compound semiconductors such as Group III antimonides, arsenides and phosphides. Phosphides of gallium and indium have been prepared in the form of single crystals [6,7] for use as electroluminisecents and high temperature rectifiers. Similar properties are expected for the phosphides of boron and aluminium. The present study was undertaken to determine the heats of formation of the said phosphides which may provide an insight into the structural characteristics of these compounds. Zinc phosphide was also included in this study to compare the value of its heat of formation with the reported values obtained by others.

MATERIALS AND METHODS

Reagents. Boron phosphide and aluminium phosphide were prepared in the laboratory by the methods of Ruehrwein [8] and Addamiano [9] respectively using the reactions:

$$AlP + BCl_3 \xrightarrow{1000^o} BP + AlCl_3$$

$$2Al + Zn_3P_2 \xrightarrow{800^o} 2AlP + 3\,Zn$$

$AlCl_3$ and Zn sublimed off at the temperatures of the experiments and the phosphides were left behind. The products were analysed at Materials Research Laboratories of Texas Instruments Inc., Dallas, U.S.A. The spectroanalysis results reported the impurities less than 0.1% in each case.

Purified zinc phosphide powder (Fisher Scientific Co., U.S.A), was resublimed in an inert atmosphere and then used for all purposes.

Purified amorphous boron and aluminium powder were supplied by Fisher Scientific Co., and Denver Fire Clay Co., U.S.A., respectively.

Amorphous, technical grade red phosphorus powder, granular zinc and sodium carbonate, both analytical grade, were all supplied by J.T. Baker Chemical Co., U.S.A. All these reagents met ACS specifications.

Benzoic acid used in the standardisation of the calorimeter, was supplied by Parr Instrument Co., Illinois, U.S.A. It was of calorimetric grade with reported heat of combusion as 6318 cal/8.

Commercially available compressed oxygen furnished by Denver Oxygen Co., U.S.A., was used for firing the samples.

Equipment. The heat of combustion measurements were made on Emerson Fuel Calorimeter, adiabatic type. Adiabaticity was achieved by a thick felt lining all around

the double-walled calorimeter jacket developed by Daniels [10]. The internal capacity of the special acid resistant stainless-steel bomb was 500 ml and it was filled with oxygen at a pressure of 300 psi each time before ignition. The bomb was fitted with a loop electrode to hold the combustion cup. The calorimeter can made of thin sheet metal, had a capacity to hold 3250 ml water.

Throughout the experiments, glazed silica cups were used to hold the samples instead of metal cups which melted due to excessive heat liberated during the combustion.

Standard iron wire of 0.004 in dia was used as ignition wire with a heat of combustion as 1600 cal/g. Measured 8–12 cm length of the wire was coiled in the form of a spiral, ends of which were connected to the electrodes and the central loop touched the sample in the silica cup.

The combustion was achieved by short circuiting the fuse wire with the help of a resistance type ignition circuit. Approximately 35 ohms resistance and a current of 1.7 amperes were required.

The temperature rise in the calorimeter was measured with a Fisher calorimeter thermometer, 24 in long with a range of 18–28° (subdiv 0.001°). The temperatures observed with a thermometer reading lens were corrected with Master Standards certified by National Bureau of Standards, Washington, D.C. The temperature of the calorimeter jacket was recorded with an 18-in calorimeter thermometer having a range of 15–35° with subdiv. 0.05°.

Procedure

(a) *Standardisation.* It implies the operation of the calorimeter with a standard sample from which the energy equivalent of the system could be calculated. This factor includes the heat capacity of water bucket, water itself, bomb and its contents, dipped parts of the thermometer, stirrer and supports for the water bucket.

Standardisation of the calorimeter was done each time before a fresh sample was used. The method recommended by Parr [11] was employed. 5–7 parallel measurements were made for each standardisation. One g of powdered benzoic acid was compressed into a pellet for each measurement and it was weighed before use. The calorimeter bucket was filled with weighed 1950 g of water for every determination.

(b) *Ignition of Phosphide Samples.* This was carried out in the same manner as the standardisation except that the benzoic acid pellets were replaced by finely powdered phosphide samples. The oxygen pressure in the bomb was built up very slowly to avoid sweeping away of the samples.

After the run was made, the solid firing products were collected for subsequent analyses. The gaseous products were allowed to escape.

(c) *Ignition of the Mixtures.* Exactly 1 g of a mixture of phosphorus and metal dust was weighed. The mixture contained the exact stoichiometric ratio of the elements to give a particular phosphide. The elements were intimately mixed and the weighed sample was ignited under identical conditions as used in (b). The combustion products were retained for analysis as before.

(d) *Analysis of the Combustion Products.* The solid products obtained from the combustions above were subjected to qualitative analysis on a General Electric XRD-5 X-ray diffraction unit with spectrogoniometer. The dried products were also studied as nujol mull on a Beckman IR-5 spectrophotometer using very low concentration to get better peaks.

Calculations

The heat of formation of metal phosphides was computed from the combustion data on the basis of the following scheme of reactions in which Me stands for metal and P for phosphorus:-

$$Me_{(s)} + P_{(s)} \longrightarrow MeP_{(s)} + \Delta H_1 \quad (1)$$

$$MeP_{(s)} + O_{2(g)} \longrightarrow \text{Products} + \Delta H_2 \quad (2)$$

$$Me_{(s)} + P_{(s)} + O_{2(g)} \longrightarrow \text{Products} + \Delta H_3 \quad (3)$$

Considering the products to be the same in reactions (2) and (3), reaction (1) results by subtraction of (2) from (3):

$$Me_{(s)} + P_{(s)} \longrightarrow MeP_{(s)} + \Delta H_3 - \Delta H_2 \quad (4)$$

Thus $\Delta H_1 = \Delta H_3 - \Delta H_2$

By definition, $\Delta H_3 = \Delta \bar{H}_{\text{comb. (Me+P)}}$

$$\Delta H_2 = \Delta \bar{H}_{\text{comb. (MeP)}}$$

$$\Delta H_1 = \Delta \bar{H}_{\text{f (MeP)}}$$

Therefore equation (4) may be rewritten as

$$\Delta \bar{H}_{\text{f (MeP)}} = \Delta \bar{H}_{\text{comb. (Me+P)}} - \Delta H_{\text{comb. (MeP)}} \quad (5)$$

Equation (5) on per gram basis is expressed as

$$\Delta H_{\text{f (MeP)}} = \Delta H_{\text{comb. (Me+P)}} - \Delta H_{\text{comb (MeP)}}$$

RESULTS AND DISCUSSION

The analysis of the combustion products revealed beyond any doubt that the end-products of the ignition

Table 1. Heats of formation of phosphides of Zn (II), Al (III) and B (III) at 298°K

Compound or mixture	Energy equivalent of calorimeter (cal/°C)	Heat of combustion (kcal/g) ΔH_{comb}	Difference in the heats of combustion (kcal/g) $\Delta H_f = \Delta H_{(Me+P)} - \Delta H_{(MeP)}$	Heat of formation (kcal/mole) ΔH_f
Zinc phosphide, Zn_3P_2	2576 ± 20	− 2.35 ± 0.01		
			− 0.35 ± 0.032	− 90.3 ± 8.3
Zinc+phosphorus	2577 ± 22	− 2.70 ± 0.03		
Aluminium phosphide, AlP	2579 ± 35	− 5.67 ± 0.01		
			− 0.50 ± 0.022	− 29.0 ± 1.3
Aluminium+phosphorus	2605 ± 34	− 6.17 ± 0.02		
Boron phosphide, BP	2562 ± 17	− 7.42 ± 0.04		
			− 1.44 ± 0.081	− 60.2 ± 3.4
Boron + phosphorus	2576 ± 19	− 5.98 ± 0.07		

of phosphide samples and metal–phosphorus mixtures were identical. Further the extent of combustion and the amount of products obtained were also identical. As such, the heats of formation of the phosphides could be calculated as a difference between the heats of combustion of the phosphides and the metal–phosphorus mixtures in which the elements were present in stoichiometric ratios [12]. The heats of combustion data for each sample are reported as an average of 5 determinations and the precision is given in the form of standard deviation (Table 1). Heats of formation of individual phosphides as obtained in this study are discussed below:

Zinc Phosphide, Zn_3P_2. The value obtained in the present investigation agrees well with that reported by Schukarev *et al*. [13] who found it to be − 98 ± 3 kcal/mole from the heat of combustion of zinc phosphide. Karvelis [4] has also reported a value, $\Delta H_{298^\circ} = 55.03 \pm 1.5$ kcal, for the same phosphide which is considerably less than our value. Karvelis used a similar technique as is under report but his value was calculated only from one combustion using the reaction between zinc dust and red phosphorus in an adiabatic calorimeter. The reported yield of zinc phosphide was 53–67% at 360–420°K. The low value of heat of formation obtained by Karvelis is obviously due to incomplete reaction between zinc and phosphorus and may also be the result of some side reactions yielding products other than zinc phosphide. These products are not unexpected to be formed at the high temperatures which develop during calorimetric combustions. In the present study, complete combustion was obtained due to better technique and high efficiency of the calorimeter. Further our value has been calculated as a difference between two large values of heats of combustion which eliminates the errors due to possible side reactions which are expected to be similar because of identical conditions of experiment. As such their effects would cancel out. Agreement with Schukarev *et al*. suggests that the basis of calculations for our value is also valid and sound.

Aluminium Phosphide, AlP. The heat of formation found in this study is not conclusive because very few studies have been made for this compound. A value of −28.2±3.2 kcal/mole for its heat of formation has been reported earlier by De Maria *et al*. [14]. They obtained this value theoretically from the 2nd and 3rd law enthalpies for the reaction, $4\ Al_{(g)} + Al_2O_{3(s)} = 3\ Al_2O_{3(g)}$, combined with the enthalpy of decomposition of aluminium phosphide,

$$2\ AlP_{(s)} = 2\ Al_{(g)} + P_{2(g)}$$

at the same temperature.

Experimental value found by us is quite consistent with the observed properties of aluminium phosphide reported by White and Bushey [15]. This compound is very stable and resistant to fusion and vaporisation in the absence of moisture. The value under report would predict such properties for this compound.

Boron Phosphide, BP. Heat of formation of boron phosphide, $\Delta H_{298^\circ} = + 60 \pm 5$ kcal, obtained in this study appears very unusual considering the stability of this compound at ordinary temperatures. Phillip, Hayman and Stuart [16] found $\Delta \overline{H}_{f(298^\circ)} = -27.6 \pm 1.1$ kcal for it. They determined it calorimetrically by comparing the heat of combustion of B phosphide in F with that of stoichiometric mixtures of beta-rhombohedral boron and white phosphorus. Another value of −59.15 kcal/mole at 298°K has been found by Gardner [17].

Boron phosphide employed in the present study was identified by X-ray diffraction as metastable hexagonal

close-packed crystalline variety. This form undergoes a very slow transformation to the face-centered cubic form at room temperature. The relatively high value for the heat of formation of BP(H.C.P.) may be the result of a different mechanism of the reactions taking place during the ignition of the BP and (B + P) mixtures. This scheme of reactions is given below:

$$B_{(s)} + P_{(red)} + O_{2(g)} \longrightarrow \Delta H_{f(BP)} + B_2O_{3(s)} + P_2O_{5(s)} + \Delta H_1$$

$$BP_{(s)} + O_{2(g)} \longrightarrow B_2O_{3(s)} + P_2O_{5(s)} + \Delta H_1$$

$$\longrightarrow BPO_{4(s)} + \Delta H_2$$

$$\Delta H_{comb\ (B+P)} = \Delta H_{f(BP)} + \Delta H_1$$

$$\Delta K_{comb\ (BP)} = \Delta H_1 - \Delta H_2$$

$$\Delta H_{comb\ (B+P)} - \Delta H_{comb.(BP)} = \Delta H_{f(BP)} + \Delta H_2$$

If ΔH_2 in the above equation is much larger than $\Delta H_{f(BP)}$, the observed value of heat of formation of BP would be +ve. ΔH_2 is the heat of formation of boron phosphate from boron oxide and phosphorus pentoxide. This value is not available in the literature. As such the postulated mechanism as above cannot be verified conclusively.

The combustion products obtained from the firing of BP and B+P mixture were amorphous in nature. Similar products were obtained by Dworkin, Sasmore and Van Artsdalen [18] in their attempt to determine the heat of combustion of boron nitride calorimetrically. The amorphous nature of the products refused to yield any reliable information regarding their nature and extent of production.

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MICROBIAL CHEMISTRY

Part III. Isolation and Identification of the Metabolic Products of *Penicillium funiculosum* Thom*. The Chemistry of Funiculosic Acid

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Penieillium funiculosum Thom, grown on a semisynthetic medium has been shown to produce diethyl phthalate and a phthalaldehydic acid, $C_9H_8O_5$, for which the name funiculosic acid has been proposed. The chemistry of funiculosic acid (II) has been investigated and its synthesis achieved.

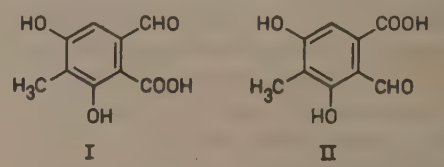
INTRODUCTION

During screening of indigenous mycoflora, a strain of *Penicillium* series was isolated by one of us (N.M.) which was identified and confirmed as *Penicillium funiculosum* Thom (IMI-157575). In the earlier reports this mould has been shown to produce helenine [1], mitorubrin [2] and related compounds, funiculosin [3], funicone [4] and deacetoxywortmannin [5].

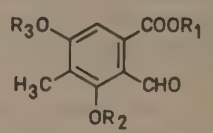
In the present studies, the culture filtrate of *Penicillium funiculosum*, grown on Czapeck-Dox medium enriched with carrot extract [6] was extracted with ethyl acetate. The extract was then separated into acidic and neutral fractions. The neutral fraction furnished an oily product with pleasant smell, b.p. 295°. If analysed for $C_{12}H_{14}O_4$. In the IR spectrum it showed bands at ν_{max} 1723 (C=O, ester), 1600, 1580 cm^{-1} (aromatic stretching vibrations). It gave a positive hydroxamic acid test for esters and on alkaline hydrolysis furnished phthalic acid, m.p. 206° (dec). The neutral oily product was, therefore, identified as diethyl phthalate, confirmed by superimposed IR spectrum with that of authentic sample. Isolation of phthalic acid esters from microbial sources is not very common. However, in an earlier report Cross *et al.* [7] have described the isolation of dimethyl phthalate from *Gibberella fugikuroi*.

From the acidic fraction a crystalline acid, m.p. 260° (dec) was obtained for which the trivial name funiculosic acid has been proposed.

Funiculosic Acid. Funiculosic acid, obtained as yellow rods from n-propanol–water m.p. 260° (dec), is optically inactive. It analysed for $C_9H_8O_5$ (M^+ 196). The proposed structure II follows from its chemical degradation and spectral studies. Funiculosic acid contains one –COOH group. A positive silver mirror test and formation of 2,4-DNP was indicative of the presence of –CHO group. A violet to red colouration with ferric chloride was compatible with a *meta*-diphenol system. The PMR spectrum of funiculosic acid revealed the presence of a methyl group attached to a benzene ring, δ 2.05 (3H, s. Ar-CH_3) and only one aromatic proton δ 7.1 (1H, s.).



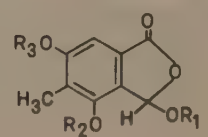
I II



III $R_1=CH_3$ $R_2=R_3=H$

IV $R_1=C_2H_5$ $R_2=R_3=H$

V $R_1=C_2H_5$ $R_2=R_3=Ac$



VI $R_1=R_2=R_3=H$

VII $R_1=R_2=R_3=Ac$

VIII $R_1=H$ $R_2=R_3=CH_3$

IX $R_1=R_2=R_3=CH_3$

Esterification of funiculosic acid ($EtOH/H_2SO_4$) afforded the corresponding ethyl ester (IV), m.p. 165°. Acetylation of IV with acetic anhydride–pyridine furnished O,O-diacetyl derivative V, $C_{15}H_{16}O_7$, m.p. 92°. However, when funiculosic acid was acetylated under similar

*Pakistan J. Sci. Ind. Res. 19, 120 (1976) may be regarded as Part II of the series.

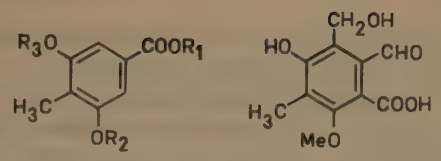
condition, instead of the expected diacetoxy derivative, a triacetate VII $C_{15}H_{14}O_8$ (M^+ 322) m.p. 195° was obtained. The IR and PMR spectra of VII showed absence of –CHO and –COOH groups. The presence of absorption bands at ν_{max} 1789 cm^{-1} (C=O, lactol) and particularly the signal due to the methine proton –O–C*H*–OAc appearing at δ 7.4 (1H,*s*) in the PMR spectrum were compatible with structure VII.

Similarly methylation of funiculosic acid with methyl iodide and potassium carbonate afforded an O,O-dimethyl derivative VIII, m.p. 173° and an oily product believed to be IX. However, due to scarcity of material IX could not be purified and hence was not studied further. The IR. spectrum of VIII showed absence of –CHO and –COOH groups. The presence of bands at ν_{max} 3401 (–OH) and 1764 cm^{-1} (C=O, lactol) were compatible with structure VIII.

The formation of lactols VII and VIII implies that the –CHO and –COOH groups are located *ortho* to each other. Also there exists strong intramolecular hydrogen bonding between the –CHO and the adjacent phenolic –OH in funiculosic acid, II, which inhibits the formation of lactol and accounts for the stability of the molecule. Removal of hydrogen bonding in II by acetylation or methylation favours the formation of lactol derivatives VII and VIII, respectively.

The reaction of aldehydes with fused alkali resulting in disproportionation or cleavage has been successfully employed in structural elucidation of natural products. Birkinshaw and his colleagues [8] reported the isolation of 3,5-dihydroxy-p-toluic acid X by reacting cycloplic acid XIII with fused alkali. Similarly in our hands funiculosic acid on reaction with fused alkali also furnished 3,5-dihydroxy-p-toluic acid X The later compound proved very useful since, with the exception of –CHO group, it clearly indicated the relative positions of all the substituents in the the benzene ring of funiculosic acid. Placing the remaining formyl function *ortho* to the carboxyl group will give structure II for funiculosic acid. However, the evidence elaborated so far can not exclude the possibility of the alternate structure I, because on reaction with fused alkali it would also give X as in the case of cycloplic acid XIII.

Structurally related to I and II, are the aldehydic acids XV and XVI, reported by Birkinshaw and his coworkers [9] as the selective oxidation products of quadrilineatin XIV. On reduction followed by methylation, XV furnished 4,6-dimethoxy-5-methyl phthalide XVII, m.p. 157°, while the isomeric acid XVI afforded 5,7-dimethoxy-6-methyl phthalide. XVIII, m.p. 172°. Both the phthalides have been independently synthesised by Birch *et al.* [10].

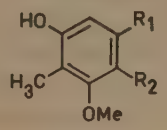


X $R_1=R_2=R_3=H$

XI $R_1=CH_3$ $R_2=R_3=H$

XII $R_1=H$ $R_2=R_3=CH_3$

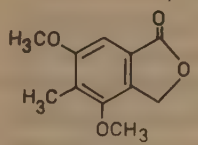
XIII



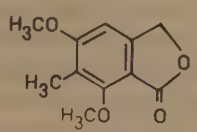
XIV $R_1=R_2=CHO$

XV $R_1=COOH$, $R_2=CHO$

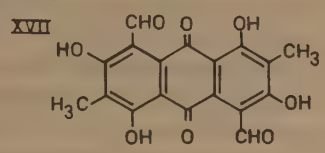
XVI $R_1=CHO$, $R_2=COOH$



XVII



XVIII



XIX

Similarly the O,O,-dimethyl derivative, 4,6-dimethoxy-3-hydroxy-5-methyl phthalide VIII on reduction with zinc – sulfuric acid furnished a compound m.p. 156° identified as phthalide XVII and confirmed by direct comparison with an authentic sample of XVII prepared by the action of chloromethyl ether on XII according to the procedure of Birkinshaw *et al.* [9]. The formation of 4,6-dimethoxy-5-methyl phthalide XVII suggests that funiculosic acid is a derivative of *para*-toluic acid and hence structure II was assigned to it. Incidently Sassa *et al.* [11] reported isolating from an unidentified mould a methyl ester $C_{10}H_{10}O_5$ which on alkaline hydrolysis afforded an acid m.p. 260° (dec) identical with our funiculosic acid.

Funiculosic acid when heated above its melting point dimerises to a reddish compound, λ_{max} 535 nm which gives a reddish colour in alkali and an orange colour in sulfuric acid. The dimer is believed to have structure XIX which is consistent with the observation of Mody and Shah [12].

Synthesis of Funiculosic Acid. The key step in the synthesis of funiculosic acid is the formylation of 3,5-dihydroxy-*p*-toluic acid methyl ester XI which was obtain-

ed from *p*-toluic acid through disulfonation [13] followed by alkali fusion [14] and then esterification.

Formylation of phenolic compounds has been accomplished conveniently employing the Reimer–Tiemann or Gattermann reaction. In our hands when XI was treated with $CHCl_3$–NaOH, under Reimer–Tiemann conditions, a coloured resinous residue was obtained which could not be crystallised. However, reaction of XI with Zn $(CN)_2$–$AlCl_3$–HCl (dry) according to Gattermann's procedure readily furnished the corresponding 2-formyl derivative, identical with methyl-funiculosate III, which on hydrolysis afforded funiculosic acid II, m.p. 260° (dec) identical with the sample isolated above as the metabolic product of *Penicillium funiculosum.*

EXPERIMENTAL

Melting points are recorded on Kofler block and are uncorrected. UV spectra were measured on a Beckmann model D.B. spectrophotometer in methanol. IR spectra were recorded on a Perkin–Elmer 137 spectrophotometer in KBr, unless otherwise stated. PMR spectra were taken in d_6-DMSO on a Varian A-60 spectrophotometer. Chemical shifts are given in ppm relative to internal reference TMS: (d,doublet; q, quartet; s,singlet; t,triplet). The mass spectra were measured on A.E.I.-MS9 instrument at 70 eV. TLC was carried out on silica gel-PF254. The petroleum ether used refers to the fraction with b.p. 60–80°.

Organism. Penicillium funiculosum Thom was isolated by one of us (N.M.) from the Karachi soil and was confirmed by the Commonwealth Mycological Institute, England and catalogued under No: IMI-157575.

Penicillium funiculosum was first inoculated on ordinary Czapeck-Dox medium in test tubes and incubated at 27° for 9 days. The nine-day old culture was then used to inoculate flasks containing the culture medium.

Culture Conditions. The modified Czapeck-Dox medium used was composed of glucose, 50.0 g; $NaNO_3$, 3.0g; KH_2PO_4 1.0 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeSO_4.7H_2O$, 0.01 g and aqueous carrot extract [6], 1 liter.

In a typical batch 50 (1 l) conical flasks containing 350 ml of the above medium were autoclaved at 10 lb pressure for 20 min then inoculated with the nine-day tube culture of *Penicillium funiculosum* till there was no more consumption of glucose, determined polarimetrically. The mycelia were separated by filtration and the broth (13.8 l; pH 6) was repeatedly extracted with ethyl acetate. The combined extracts were dried (an Na_2SO_4), concentrated under reduced pressure and extracted with sodium bicarbonate solution. The neutral ethyl acetate layer (Fraction A) and the alkaline aqueous layer (Fraction B) were worked up separately.

Isolation of Diethyl phthalate. The neutral ethyl acetate layer (Fraction A) was evaporated to dryness under reduced pressure and the resulting oily residue was thoroughly extracted with petroleum-ether by refluxing. The combined extracts were washed with dil sodium bicarbonate solution, then with water and finally dried (an Na_2SO_4). Removal of sovlent furnished diethyl phthalate as pleasant smelling oil (300 mg), b.p. 295°. ν_{max} 1723 (C=O, ester), 1600, 1580 cm^{-1} (aromatic). (Found: C 64.55, H 6.4%. $C_{12}H_{14}O_4$ requires: C 64.85, H 6.35%).

Hydrolysis: Diethylphthalate (100 mg) in ethanol (5 ml) was added to a solution of KOH (140 mg) in ethanol (2.5 ml) and the mixture refluxed for 2 hr. On usual work-up, it afforded white crystals of phthalic acid, (25 mg) m.p. 206° lit [7] m.p. 212–213°.

Isolation of Funiculsoic Acid. The alkaline aqueous layer (Fraction B) was acidified (pH 1) with 2*N*HCl and extracted with ethyl acetate. The organic layer was washed with water, dried (an Na_2SO_4), and solvent removed. The resulting solid on crystallisation from n-propanol–water, gave yellow rods of *funiculosic acid,* (600 mg), m.p. 260° (dec) λ_{max} 225 (log ϵ 4.314), 290 nm (log ϵ 4.302). ν_{max} 3425 (–OH, phenolic), 1695 (C=O, aldehyde and carboxyl groups), 1626, 1587 cm^{-1} (aromatic). δ 2.05 (3H, s, Ar-CH_3); δ 5.6 (1H, broad –COO*H*), δ 7.1 (1H, s, aromatic), δ 10.4 (1H, s, –C*H*O), δ 12.8 (2H, broad singlet 2× –O*H* phenolic); *m/e* 196 (M^+), 178, 168, 167, 150 (base peak), 123, 122. (Found: C 54.76, H 4.1%. $C_9H_8O_5$ requires: C 55.11, H 4.11%).

Funiculosic acid is soluble in methanol, ethanol, ethyl acetate and acetone; sparingly soluble in benzene, ether and hot water; insoluble in chloroform and petroleum ether.

Methyl Funiculosate (III). Funiculosic acid (100 mg), methanol (10 ml) and concd sulfuric acid (0.1 ml) were refluxed on a water-bath for 5 hr. On working up in the usual manner and crystallisation of the resulting solid from benzene furnished methyl funiculosate III (40 mg), m.p. 170° (M^+ 210), ν_{max} 3400 (–OH), 1680 (C=O, ester), 1638 cm^{-1} (C=O, aldehyde), λ_{max} 230, 250 and 299 nm. δ 2.36 (3H, s, aromatic CH_3), δ 3.77 (3H, s, –CO–OCH_3) δ 11.56 (1H, s, C*H*O), δ 14.0 (2H, broad, 2× –O*H*).

Ethyl Funiculosate (IV). Funiculosic acid (50 mg), ethanol (5 ml) and concd sulfuric acid (0.1 ml) were refluxed on a water-bath for 4 hr. On working up in the usual manner crystallisation of the gummy solid from aqueous ethanol furnished ethyl funiculosate IV, (25 mg) m.p. 165°, ν_{max} 3165 (–OH). 1718 (C=O, ester); 1706 cm^{-1} (C=O, aldehyde). δ 1.4 (3H, t, *J* 7.5 Hz, CH_3CH_2). δ 2.1 (3H, s, Ar-CH_3); δ 4.35 (2H, q, *J* 7.5 Hz, –CH_2–CH_3); δ 7.04 (IH, s, aromatic); δ 10.32 (1H, s, –C*H*O). δ 12.82

(2H, s, 2x –O*H*), *m/e* 224 (M^+), 196, 195 (base peak), 179, 177, 151, 150, 149, 123, 122, 121.

Ethyl O,O-Diacetyl Funiculosate (V). A solution of ethyl funiculosate (IV, 25 mg) in acetic anhydride (2.5 ml) and pyridine (2.5 ml) was refluxed for 2 hr and worked up in the usual manner to give ethyl O,O-diacetyl funiculosate. (V, 20 mg), m.p. 92° (from benzene–petroleum ether). ν_{max} 1754 (–OCOCH_3); 1706 cm^{-1} (C=O, ester and aldehyde). (Found: C 58.24. H 5.51%. $C_{15}H_{16}O_7$ requires. C 58.44, H 5.23%).

3,4,6-Triacetoxy-5-methyl Phthalide (VII). A solution of funiculosic acid (II, 50 mg), acetic anhydride (5 ml) and pyridine (5 ml) was allowed to stand at 37° for 6 days. The reaction mixture was then poured into ice-water and the solid obtained on crystallisation from benzene–petrleum ether afforded 3,4,6-triacetoxy-5-methyl phthalide (VII, 40 mg), m.p. 195°. λ_{max} 280, 270, 224 nm. ν_{max} 1789 (C=O, lactol); 1751 cm^{-1} (O-COCH_3); δ 2.13 (6H, s, Ar-CH_3 and OCOCH_3); δ 2.36 (6H, s, 2x OCOCH_3); δ 7.4 (1H, s –OC*H*OAc), δ 7.53 (1H, s, aromatic proton) *m/e* 322 (M^+), 262, 236, 220, 219, 196, 195, 194, 180, 179 (base peak), 151, 150, 84. (Found: C 55.99, H 3.9%. $C_{15}H_{14}O_8$ requires: C 55.90, H 4.38%).

4,6-Dimethoxy-3-hydroxy-5-methyl Phthalide (VIII). A mixture of funiculosic acid (II, 650 mg), anhydrous potassium carbonate (4.25 g), methyl iodide (4 ml) and dry acetone (50 ml) was refluxed for 24 hr. Acetone was removed under reduced pressure, the residue was diluted with water and extracted with ether. The aqueous phase on acidification with 2*N* HCl, furnished O,O-dimethyl funiculosic acid (VIII, 200 mg), m.p. 173° (from methanol–water). ν_{max} 3401 (–OH); 1764 (C=O, lactol); 1629 and 1587 cm^{-1} (aromatic). (Found: C 58.64, H 5.3% $C_{11}H_{12}O_5$ requires: C 58.8 H 5.35%).

Reduction of O,O-Dimethyl Funiculosic Acid (VIII). To a solution of O,O-dimethyl funiculosic acid (VII, 100 mg) in methanol (2.5 ml) was added zinc dust (200 mg) and sulfuric acid (50% v/v, 1.5 ml) and warmed for 15 min. The solution was filtered and the filtrate was diluted with water, chilled and the resulting solid crystallised from ethanol to give colourless needles of 4,6-dimethoxy-5-methyl phthalide (XVII, 25 mg), m.p. 154°; lit [10] m.p. 157°. λ_{max} (EtOH) 217 (log ε 4.537), 255 (log ε 3.834) and 296 nm (log ε 3.449); ν_{max} 1727 (C=O, lactol), 1629, 1577 cm^{-1} (aromatic).

Alkali Fusion of Funiculosic Acid. Potassium hydroxide (2.5 g), water (0.5 ml) were heated to 150° (under nitrogen) in a nickel crucible and to it funiculosic acid (500 mg) was added during 15 min. The bath temperature was raised and maintained at 310–320° for 20 min. The brownish melt was cooled, diluted with water (50 ml) and after acidification (pH 2) with 2*N* HCl, calcium chloride (200 mg) dissolved in water (2 ml) was added and the solution was allowed to stand overnight. The precipitated calcium oxalate was filtered off and the filtrate was extracted with ether. Removal of solvent and sublimation of the residue afforded 3,5-dihydroxy-*p*-toluic acid (200 mg), m.p. 240–45° (dec.); lit [14]. m.p. 245–50° (dec).

Preparation of 4,6-Dimethoxy-5-methyl Phthalide (XVII). 3,5-Dimethoxy-*p*-toluic acid [14] (100 mg), glacial acetic acid (1.5 ml) and chloromethyl ether (1 ml) were heated on water bath for 10 hr. After addition of 1*N* HCl (25 ml), the reaction mxiture was refluxed for another 1 hr. The resulting solid on crystallisation from ethanol furnished white needles of 4,6-dimethoxy-5-methyl phthalide (XVII, 20 mg), m.p. 156° (lit [10] m.p. 157°).

Preparatioon of Methyl-3, 5-dihydroxy-p-toluate (XI). 3,5-Dihydroxy-*p*-toluic acid (500 mg), methanol (25 ml) and concd sulfuric acid (0.25 ml) were refluxed under anhydrous conditions for 6 hr. On working up in the usual manner, a solid was obtained which on crystallization from benzene furnished methyl-3, 5-dihydroxy-*p*-toluate (XI, 170 mg), m.p. 188° (lit [8] m.p. 189–190°).

Formylation of (XI). Into a 2-necked (50 ml) round-bottomed-flask, kept in freezing mixture and fitted with a reflux condenser and a gas bubbler, were added methyl-3, 5-dihydroxy-*p*-toluate (XI) [9,14] 100 mg) dissolved in dry ether (10 ml) and freshly prepared zinc cyanide (150 mg). The reaction mixture was stirred magnetically and to it was added aluminium chloride (150 mg) in chilled dry ether (25 ml). The dry hydrogen chloride gas was then bubbled through this mixture for 45 min. The reaction mixture was then allowed to stand overnight, the supernatent liquid was decanted off and to the pasty residue, water (2 ml) was added and the mixture was boiled for 5 min. On cooling, a solid was separated which on crystallisation from benzene gave methyl-2-formly-3, 5-dihydroxy-*p*-toluate (30 mg), m.p. 170° identical with methyl funiculosate III.

Hydrolysis. Methyl-2-formyl-3,5 -dihydroxy-*p*-toluate (III, 15 mg) and 1*N* NaOH solution (0.2 ml) were kept together for 20 hr at room temperature. The reaction mixture was diluted with water (5 ml), acidified and extracted with ethyl acetate. Removal of solvent and crystallisation of the solid obtained from n-propanol–water furnished 2-formyl-3, 5-dihydroxy-*p*-toluic acid (8 mg), m.p. 260° (dec) identical with funiculosic acid (II).

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DIFFERENTIAL THERMAL ANALYSIS OF TETRAALKYLAMMONIUM HALOBORATES

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DTA of a series of compounds of the type $R_4N^+BX_4^-$ (where R = Et or n-But and X = Cl, Br, Ph or $PhCl_2$) indicates the formation of trialkylamine trihaloboranes and dialkylamino dihaloborenes as intermediates in the formation of linear polymers instead of cyclic borazines as the end-products.

INTRODUCTION

Several attempts have been reported in the literature [1–3] to obtain boron–nitrogen linear polymers in preference to the formation of small ring compounds. The paration of linear polymers was considered interesting because it is expected that such linear polymers will be chemically and thermally more stable compared to small ring compounds[4–6]. It was, therefore, decided to substitute different tertiary alkyl groups in place of primary or secondary alkyl groups in tetraalkylammonium haloborates $[R_4N^+BX_4^-]$ in the hope to obtain linear boron–nitrogen polymers instead of small ring compounds on pyrolysis. This paper investigates the comparative differential thermal analysis study of tetraalkylammonium trichlorophenylborates and their intermediates with different alkyl groups.

EXPERIMENTAL

All the haloborates and their intermediate compounds were prepared by the known procedures [7,8]. DTA was carried out by using the Standate model 6–25 (Stantan Instruments, England). For these experiments sample sizes (from 0.1 to 0.15 g) were used alongwith calcined alumina as the reference material. Platinum crucibles of 8 mm with 10 mm depth were found suitable. Nitrogen atmosphere was maintained over the samples at the rate of 200 ml/min throughout these experiments. Heating rates were maintained in steps varying from 2 to 16.75°/min.

RESULTS AND DISCUSSION

The compounds tetraalkylammonium trichlorophenylborates $[R_4N^+BPhCl_3^-]$, trialkylamine dichlorophenylborane $[R_3NBPhCl_2]$, trialkylamine trichloroborane $[R_3NBCl_3]$, dialkylamino chlorophenylborene $[R_2NBPhCl]$, dialkylamino dichloroborene $[R_2NBCl_2]$, B-trichloro-N-trialkylborazine $[1/3\ (RNBCl)_3]$, B-triphenyl-*N*-trialkylborazine $[1/3\ (RNBPh)_3]$, were subjected to DTA showing interesting results (Fig. 1 showing DTA curves).

When R in $[R_4N^+BPhCl_3^-]$ is ethyl it undergoes decomposition giving endothermic peak at 225°. On raising the temperature decomposition proceeds to triethylamine dichlorophenylborane and not triethylamine trichloroborane. When tetraethylammoniumtrichlorophenylborate and triethylamine dichlorophenylborane are subjected to DTA a common volatilization endothermic peak at 268° corresponding to the diethylamino chlorophenylborene is obtained. This, therefore, confirms that during the decomposition, diethylamino dichloroborene has no part to play as an intermediate with DTA of tetraethylammonium trichlorophenylborate. Further increase in the temperature

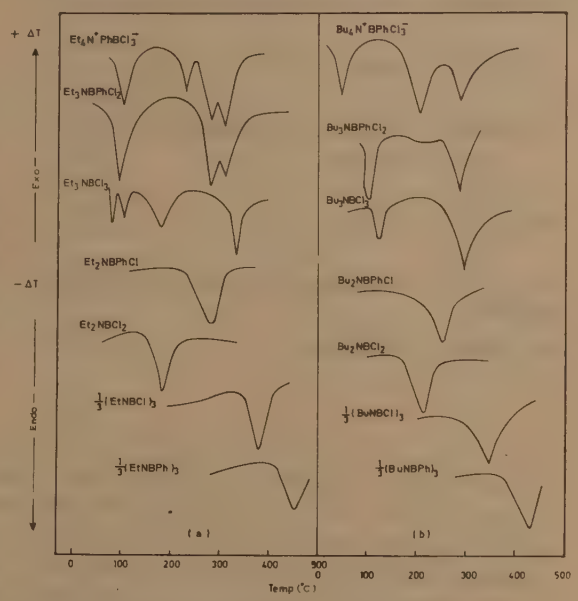

Fig.1 DTA of tetraalkylammonium haloborates.

of the sample containing tetraethylammonium trichlorophenylborate, triethylamine dichlorophenylborane,triethylamine trichloroborane and diethylamine chlorophenylborene, diethylamino dichloroborene gave no indication of cyclic borazines formation because of the absence of their characteristic endothermic peak (DTA peak for B-trichloro-*N*-triethylborazine is at 365° and B-triphenyl *N*-triethylborazine is at 425°).

When R is n-butyl in $[R_4N^+BPhCl_3^-]$ tetra-n-butylammonium trichlorophenylborate exhibits an intense volatilization endothermic peak at 235°. Further increase in the temperature results in the decomposition to tri-n-butylamine dichlorophenylborane and/or tri-n-butylamine trichloroborane. This is because two peaks are so close to each other that their differentiation from each other is extremely difficult. Similar to ethyl system a common volatilization endothermic peak corresponding to the formation of di-n-butylammino dichloroborene is obtained. When tetra-n-butylammonium trichlorophenylborate and tri-n-butylamine trichloroborane are subjected to DTA, peak corresponding to di-n-butylamino dichloroborene is observed. Tetra-n-butylammonium trichlorophenylborates,tri-n-butylamine dichlorophenylborane, tri-n-butylamine trichloroborene , di-n-butylamino chlorophenylborene and di-n-butylamino dichloroborene on DTA studies give no indication of the formation of cyclic borazines.B-trichloro-N-tri-n-butylborazine and B-triphenyl-*N*-tri-n-butylborazine gave broad volatization endothermic peaks at 378° and 456° respectively. DTA results of tetraethylammonium haloborates, tetra-n-butylammonium haloborates and their intermediates support the following reaction sequences.

$Et_4N^+BPhCl_3^-$ System

$$Et_4N^+BPhCl_3^- \xrightarrow{(-EtCl)} Et_3NBPhCl_2 \xrightarrow{(-EtCl)} Et_2NBPhCl$$

n-$But_4N^+BPhCl_3^-$ System

$$But_4N^+BPhCl_3^- \xrightarrow{(-ButCl)} But_3NBPhCl_2 \xrightarrow{(-ButPh)} But_2NBCl_2$$

$$But_4N^+BPhCl_3 \xrightarrow{(-ButPh)}$$

$$But_3NBCl_3 \xrightarrow{(-ButCl)} But_2NBCl_2$$

Tetraethylammonium trichlorophenylborate differs from tetra-n-butylammonium trichlorophenylborate in giving different end-products on DTA. In ethyl system diethylamino chlorophenylborene is formed and in n-butyl system dibutylamino dichloroborene is formed. The preferential elimination of phenyl group instead of chloride in the n-butyl system can be easily explained on steric consideration, n-butyl groups are considerably larger than ethyl groups and in the end-product removal of phenyl group is necessitated to relieve strain in the butyl system.

Primary alkylammonium haloborates on pyrolysis leads to the formation of borazines as the end-product. Tri - and tetraalkylammonium haloborates preferentially give linear polymers of variable length. Further work is in progress to determine the exact nature of these polymers.

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ELECTRON-IMPACT INDUCED FRAGMENTATION OF 1:1 COMPLEX IN BORON–NITROGEN COMPOUNDS

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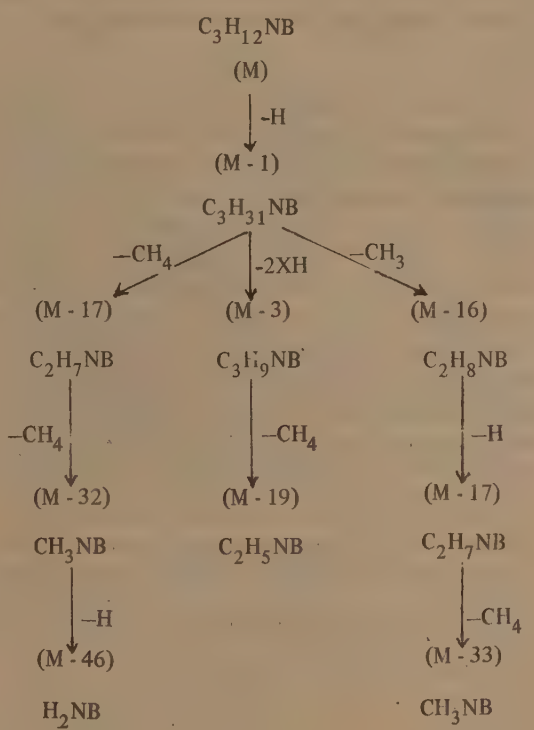
The species produced from the electron-impact induced fragmentation of 1:1 complexes of trialkylamines and haloboranes have been examined. However, when the same treatment is carried out with triethylamine borane complex some other species also appear.

INTRODUCTION

One of the interesting transformations of trialkylamine haloborane complexes ($R_3N.BX_3$: where R = Et and n-But, X = H, Cl, Br or $PhCl_2$) concerns their conversions to polyborazynes [1–4] $(RNBX)_n$. The most important method for such conversions is the direct pyrolysis of the complexes leading to the formation of dialkylamino dihaloborenes and finally to polymeric products [5].

Knowing these results we were stimulated to study the electron impact induced fragmentation of trialkylamine haloborane complexes. Mass spectra of trimethylamine haloboranes (X = Cl, Br, I and BF_nBr_m) (n + m = 3) and ethylamine haloboranes (X = H, Cl, Br and I) are already known [6,7] showing the fragmentation pattern in which mainly the cleavages of boron–nitrogen and boron–halogen bonds are indicated. Their results were interpreted in terms of bond strength. Other species detected by them are shown in Scheme 1. The dissociation process of trialkylamine haloboranes (R = Et and n-But and X = Cl, Br, $PhCl_2$) which were studied by us resembles that of trimethyl series but we noticed some additional interesting species in the fragmentation of triethylamine borane complexes.

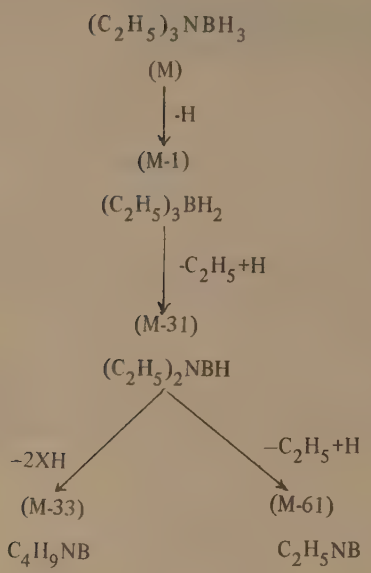
Scheme 1



RESULTS AND DISCUSSION

The mass spectra of triethylamine haloborane ($R_3N.BX_3$: R = Et and n-But, X = H, Cl, Br and $PhBCl_2$) produced from electronically induced reaction at 100° and 250° were obtained. The spectra of the complexes obtained mainly from fragmentation of R_3N and BX_3 were investigated. However, with X = H the electron induced dissociation of the complex of triethylamine borane ($Et_3N.BH_3$) leads to few other fragments as indicated from the record of fragmentation pattern given in Fig.1. The assignments of structures to these fragments were facilitated by the use of the isotopic pattern from naturally occurring isotopes of boron (B^{10} = 19.6% B^{11} = 80.4%).

Scheme 2



A kinetic mechanism to account for the observed fragmentation of (Et_3NBH_3) is proposed through a series of steps illustrated in fragmentation Scheme 2. The molecular ion M is not observed, but species corresponding to the loss of an odd number of radicals ($H^{\circ}Cr\ C_2H^{\circ}5$) from M are observed but the loss of an even number of radicals from M is not observed. The behaviour of this complex resembles that of ($Me_2NH\ BH_3$) and ($Me_3N\ BH_3$) which shows large peaks corresponding to (M–H) and (M–3H). However, in these compounds the parent ions are observed in rather low abundance and the *m/e* values are given for the ions containing B^{11}. Species arising from the further fragmentation of $(C_2H_5)_3N$ and BH_3 are not included.

EXPERIMENTAL

Mass spectra were obtained using an A.E.I., M.S.9 instrument at 70 eV and an accelerating voltage of 8 kV with the ionization chamber at 100–200°. Pure samples were introduced with a direct insertion probe.

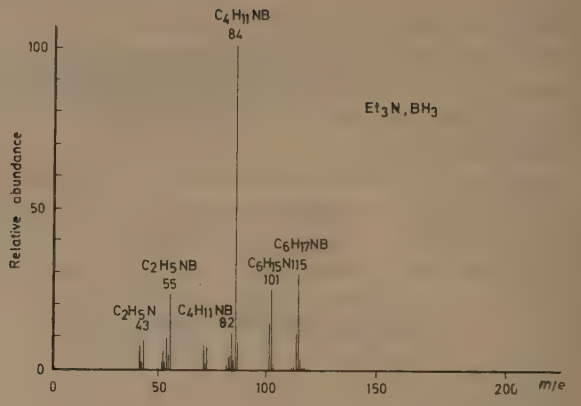


Fig. 1. Fragmentation pattern of triethylamine borane.

Triethylamine haloborane were prepared by the interaction of triethylamine and haloborane (1:1) at 80° as described previously [8].

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BIOSYNTHESIS OF ENZYMES BY SOLID SUBSTRATE FERMENTATION*

Part II. Production of Alpha-Amylase by *Bacillus subtilis*

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Production of alpha-amylase by the locally isolated cultures of *Bacillus subtilis* was studied using wheat bran as solid substrate in conical flasks. The selected culture was improved by UV irradiation. Partial replacement of wheat bran by other substrates such as rice husk, maize bran, peanut meal or penicillium-waste mycelium was also investigated for enzyme formation. Of all the substrates wheat bran was found to be an ideal for enzyme synthesis.

INTRODUCTION

In continuation of our studies regarding the use of wheat bran as solid substrate for the synthesis of cellulase [1] and amyloglucosidase [2] the present work describes the production of alpha-amylase by *Bacillus subtilis*. The wheat bran or brans of other cereals were also evaluated for enzyme formation by solid substrate fermentation. The wheat bran or brans of other cereals were also evaluated for enzyme formation by solid substrate fermentation. The surface culture or the Koji process, using trays commonly used in Japan, requires a large surface area but other factors such as rates of agitation and aeration, foaming, contamination or pH control are not serious problems in fermentation in deep tanks. Microbial amylases are extensively used for starch thinning in glucose and beer manufacture, improving dough for bread making, desizing of textile and in pharmaceutical preparations. These enzymes are imported into Pakistan involving substantial amount of foreign exchange and the largest consumer in the country is the textile industry. Alpha-amylase of bacterial origin is thermostable and thus can withstand high temperature during desizing operation in the textile industry. Much work has been done on the production of microbial amylase by surface culture process using wheat bran as a substrate [3–14]. Cultures of *Bacillus subtilis* were isolated locally from the soil and the selected strains was further improved by mutation. Enzyme formation was carried out in 1l conical flasks containing cereal brans.

MATERIALS AND METHODS

Organisms and Growth. Fifty strains of *Bacillus* species were isolated after giving heat shock to suspension of soil, wheat flour or maize products in water. The bacterial cultures were maintained on nutrient agar medium consisting of (g/l): peptone 6.0, casein (hydrolyzate) 4.0, yeast extract 3.0, glucose 2.0, beef extract 1.5 and agar 25.0. The cultures were incubated at 37° for 48 hr and then stored in a refrigerator at 5°. All isolates were gram + ve spore-forming rods which were identified as *Bacillus* species using the criteria of Bergey's Manual of Determinative Bacteriology. The culture producing maximum enzyme was further identified as *Bacillus subtilis*.

Inoculum Preparation. A vegetative inoculum was employed in the present work. 100 ml of nutrient broth, as described earlier in 1l conical flask was sterilized at 121° for 15 min. After cooling the medium, it was inoculated from the agar slant aseptically. It was allowed to grow at 30° for 24 hr on a shaker (designed and fabricated at these Laboratories, rotating at 125 rev/min).

Fermentation Procedure. A mash of 20–50 g wheat bran or the bran of other cereals was prepared by adding 2 parts of diluent phosphate buffer (1.5 g KH_2PO_4 and 3.5 g K_2HPO_4/l) or otherwise stated to one part by weight of wheat bran in 1l conical flask. The mash was sterilized at 121° for 15 min. The sterilized mash was inoculated with 2 ml of the inoculum prepared as above and incubated at 30° for 48 hr.

Culture Improvement. A 24-hr old bacterial cell suspension was centrifuged aseptically and washed with saline water twice and resuspended in 0.9% saline solution. The suspension of cells, placed in a petri dish, was placed under UV lamp. The samples were taken at different time intervals and cultivated on nutrient agar and incubated at 37°

Preparation of Enzyme Extract. The fermented bran

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cake was shaken for 1 hr at 30° on a rotary shaker after the addition of 200 ml phosphate buffer (pH 7.00) as described earlier. To this mixture was added 1 ml 20% $CaCl_2.2H_2O$ solution for each 40 ml of the buffer. The wheat bran suspension was filtered using ordinary filter paper. The clear filtrate was assayed for enzyme activity

Analytical Methods. Amylase activity was determined by the method of Fisher and Stein [15]. Enzyme solution (1 ml culture filtrate) was added to 1 ml 1% starch solution and incubated at 25° for 3 min. The reaction was terminated by the addition of 2 ml DNS (3,5-dinitrosalicylic acid) reagent (DNS, 5%). Colour due to the reducing sugar liberated was developed by heating the reactants in a boiling water bath for 5 min then rapidly cooling them to room temperature. The extinction value was determined at 550 nm spectrophotometrically using a 'spectra' by EEL Halstead Essese England.

One amylase unit liberates reducing sugar equivalent to 1.0 mg maltose hydrate under the conditions of assay. Calibration curve was also prepared using maltose 0.5 – 2.0 mg/ml.

RESULTS

Screening of Organisms. The data in Table 1 indicate the biosynthesis of alpha-amylase by the locally isolated cultures *of Bacillus subtilis* in wheat bran by the solid substrate fermentation process. The assay of enzyme was carried out 48 hr after inoculation with 24-hr old seed culture. The amount of the enzyme synthesized by the cultures No. 1, 2, 26 and 42 was 360, 370, 230 and 238 units alpha-amylase/g wheat bran respectively. For further studies, therefore, culture No. 2 was selected for the production of alpha-amylase and it was identified as *Bacillus subtilis* (PCSIR - 2).

Culture Improvement by Mutation. Bacillus subtilis PCSIR - 2 was exposed to UV irradiation. The data in Table 2 show the synthesis of alpha-amylase by *Bacillus subtilis* after irradiation for different time periods. Duplicate cultures of each isolate were examined for enzyme formation.

Culture PCSIR-29, isolated after irradiation for 40 min, produced maximum amount of alpha-amylase at 4000 units/g of wheat bran. Further irradiation did not result in any improvement in enzyme synthesis. *Bacillus subtilis* PCSIR - 29 was used for further improvement by exposing bacterial cells to 0.1 – 1.0 mg/l of 6-mercaptopurine for different time periods (Table 3). Most of the isoated colonies did not show any improvement in enzyme formation. One colony isolated (PCSIR-20) after exposing bacterial cells to the chemical for 20 hr produced 5850 units of alpha-amylase/g wheat bran. The vegetative cells of the culture (PCSIR 20) was again exposed to chemical and UV radiation ultimately

Table 1. Production of alpha-amylase, 48 hr after inoculation, by locally isolated cultures of *Bacillas subtilis* in wheat bran by surface culture method.

Isolate No.	Wheat bran (units/g)	Isolate No.	Wheat bran (units/g)
1	360	26	230
2	370	27	27
3	60	28	11
4	30	29	12.5
5	115	30	44
6	70	31	34
7	60	32	25
8	65	33	24.5
9	115	34	3.5
10	90	35	14
11	50	36	5
12	95	37	4
13	85	38	50
14	73	39	56
15	64	40	47
16	52	41	46
17	65	42	238
18	35	43	42
19	128	44	33
20	127	45	44
21	38	46	187
22	31	47	137
23	33	48	93
24	40	49	85
25	50	50	105

(Table 4). Alpha-amylase synthesis was increased as compared with the parent culture. The culture No. 6 produced maximum amount of enzyme alpha-amylase 6300 units/g wheat bran. This culture was found to be unstable and after two or three generations the enzyme formation in wheat bran substrate was about 3500–4000 units/g. The culture of *Bacillus subtilis* PCSIR - 29, isolated after UV irradiation, was stable and, therfore, it was used for further studies.

Rate of Alpha-Amylase Production. Figure 1 shows the rate of alpha-amylase synthesis by *Bacillus subtilis* PCSIR - 29. The enzyme production was determined at different time intervals after inoculation. Maximum yield of the enzyme, i.e. 4000 unit/g wheat bran, was observed at 48 hr. Further, increase in fermentation time resulted in a decrease in enzyme formation. Thus optimum time for enzyme synthesis was 42–44 hr.

Effect of Partial Replacement of Wheat Bran on Fnzyme Formation. The data in Tables 5 and 6 show the synthesis of alpha-amylase when wheat bran was partially replaced by maize bran or rice bran. Of all the substrates examined,

Table 2. Effect of UV irradiation for different time periods on the cells of *Bacillus subtilis*, PCSIR - 2 for the synthesis of alpha-amylase in wheat bran by surface culture method.

Culture No.	Time for irradiation (min)	Wheat bran (units/g)
PCSIR - 2		370
1	20	1325
2	"	1350
3	"	1500
4	"	1500
5	"	1850
6	"	1425
7	"	1700
8	"	1475
9	"	1050
10	"	1400
11	30	1900
12	"	1600
13	"	1650
14	"	1500
15	"	1800
16	"	1500
17	"	1500
18	"	1700
19	40	2100
20	"	1700
21	"	1700
22	"	1400
23	"	1400
24	"	1200
25	"	1880
26	"	2300
27	"	3500
28	"	3250
29	"	4000
30	60	1200
31	"	2000
32	"	1600
33	"	1850
34	"	1575
35	"	1725
36	"	2050
37	"	2100
38	"	2275
39	"	1270
40	"	1390

Table 3. Effect of different concentration of 6 mercaptopurine on the cells of *Bacillus subtilis* PCSIR - 29 for alpha-amylase synthesis in wheat bran substrate.

No.	Concn on mutagen (mg/ml)	Time (hr)	Wheat bran (units/g)
PCSIR - 29	—	—	4000
1	0.100	5	2500
2			1500
3			2800
4			2625
5			2375
6	0.500	5	2920
7			2175
8			1730
9			1680
10			1500
11	1.50	5	2070
12			2350
13			1825
14			2480
15	0.100	20	2950
16			3100
17			3475
18			3850
19			3525
20			5850

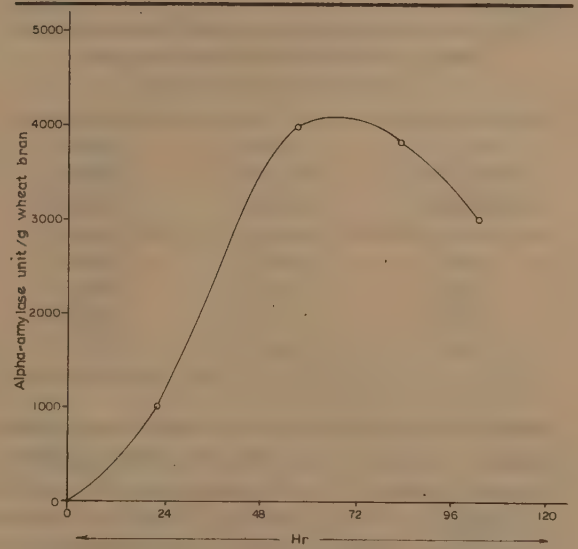

Fig. 1. Rate of alpha-amylase synthesis by *Bacillus subtilis* (PCSIR-29).

wheat bran was found to be best for the production of alpha-amylase. Maize bran was better than rice bran, since no enzyme formation was found when wheat bran was completely replaced by rice husk. The amount of enzyme produced in maize bran, however, was 2500 unit/g maize bran.

Effect of Different Nitrogen Sources on the Production of Alpha-Amylase. The biosynthesis of alpha-amylase was also investigated after partially replacing wheat bran by

Table 4. Effect of chemical treatment and UV irradiation alternatively on the the cells of *Bacillus subtilis* PCSIR - 20 for the synthesis of alpha-amylase in wheat bran substrate.

No.	Treatment			Wheat bran (unit/g)
	Chemical (mg/ml)	Time (hr)	Time for UV (min)	
PCSIR - 20	–	–	–	5850
1	0.100	20	30 min	4550
2				4775
3				4950
4				4850
5				5800
6				6300

Table 5. Effect of partial replacement of wheat bran by maize bran on the production of alpha-amylase by *Bacillus subtilis* PCSIR-29.

Wt. of wheat bran (g/flask)	Wt. of maize bran (g/flask)	Alpha-amylase (units/g)
20.0	0.0	4000
15.0	5.0	3700
10.0	10.0	3000
0.0	20.0	2500

Table 6. Effect of supplementation of rice husk to wheat bran on the production of alpha-amylase by *Bacillus subtilis* PCSIR - 29.

Wt. of wheat bran (g/flask)	Wt. of rice husk (g/flask)	Alpha-amylase (unit/g)
20.0	00.0	3800
15.0	5.0	3700
10.0	10.0	3400
00.0	20.0	00.0

peanut meal or penicillium-waste mycelium in conical flasks (Tables 7 and 8). The production of enzyme was slightly enhanced by adding 2.5 or 5.0 g peanut meal per flask. Further increase in the concentration of peanut meal resulted in the decrease of alpha-amylase formation. Moreover, rate of enzyme synthesis decreased with increasing penicillium-waste mycelium in the wheat bran cultures. Alpha-amylase production was 70% reduced when half of the wheat bran was replaced by the myelium.

The effect of the different nitrogen sources such as

Table 7. Effect of the addition of peanut meal to wheat bran substrate on the production of alpha-amylase by *Bacillus subtilis.*

Wt. of wheat bran (g/flask)	Wt. of peanut meal (g/flask)	Alpha-amylase (units/g)
20.0	00.0	3800
17.5	2.5	4200
15.0	5.0	4000
12.5	7.5	3700
10.0	10.0	2500

Table 8. Effect of the addition of penicillium-waste mycelium to wheat bran substrate on the production of alpha-amylase by *Bacillus subtilis.*

Wt. of wheat bran (g/flask)	Wt. of penicillium-waste mycelium (g/flask)	Alpha-amylase (units/g)
20.0	0.0	3800
17.5	2.5	2600
15.0	5.0	3200
12.5	7.5	2400
10.0	10.0	800

Table 9. Effect of urea and other inorganic nitrogen sources on the production of alpha-amylase by *Bacillus subtilis.*

Nitrogen source added	Alpha-amylase (units/g)
$NaNO_3$	3600
NH_4NO_3	3800
$(NH_4)_2SO_4$	3900
Urea	4200
Control	4000

$NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$ or urea (1 g N/l diluent) on alpha-amylase synthesis, was also studied (Table 9).

The production of enzyme increased slightly in the presence of urea only. Other nitrogen salts slightly decreased the enzyme synthesis.

DISCUSSION

Biosynthesis of alpha-amylase by the locally isolated culture of *Bacillus subtilis* was greatly improved by exposing the bacterial cells to UV radiation. The parent cultures

of *Bacillus species* produced 370 units/g wheat bran and, after mutation using UV radiations, the amount of enzyme synthesized was about 4000 units/g of wheat bran. The culture was further improved by exposing the cells to 6-mercaptopurine and UV radiations alternately but the culture isolated was found unstable. All the studies, therefore, were carried out using stable culture of *Bacillus subtilis* PCSIR - 29. Maize bran and rice husk were also evaluated as substrate for enzyme formation by solid substrate fermentation. Of all the substrates examined, however, wheat bran was found to be the best providing all nutrients for alpha-amylase synthesis. The addition of penicillium waste mycelium or peanut meal to the wheat bran culture medium was also investigated. Alpha-amylase formation was significantly affected when half of the wheat bran was replaced by peanut meal, of penicillin waste mycelium reduced engyme fromation. The mechanism of the inhibitory action of mycelium or peanut meal is not clear. The effect of different inorganic nitrogen sources or urea on enzyme synthesis was also studied. The addition of urea led to a slight increase in enzyme formation, the other sources of nitrogen showed on stimulatory effect.

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SOME ASPECTS OF THE BIOLOGY AND ECOLOGY OF *ZYGINA RUBRONOTATA* A PEST OF FALSA *(GREWIA ASIATICA)* IN PAKISTAN*

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Zygina rubronotata (Distant) is a serious pest of the fruit plant falsa *(Grewia asiatica)* in some areas of lower Sind in Pakistan. Studies made on different aspects of the biology and ecology of the species showed that it is usually found on falsa from September to February, with peak population during December. The life span of the species from September to February for a male ranged from 5 to 15 ($\bar{X}$ 9) days, and for females from 13 to 42 ($\bar{X}$ 27.2) days. The newly emerged adults mated and female started laying eggs within 4–6 days. In the life history studies, the average life of female, incubation period of eggs, duration of first, second, third, fourth and fifth nymphal instars were, 12.8, 7.1, 2.8, 2.5, 2.1, 1.7 and 1.4 days respectively. The oviposition period of a female was 4 – 14 ($\bar{X}$ 8) days, and the eggs laid were 16 – 44 ($\bar{X}$ 31.33).

The leafhopper affects the foliage of plants by sucking the nutrients as well as attracting growth of fungus *Phakopsora grewiae* (brown dust) in the vicinity of feeding punctures. From early September, the destruction of foliage progressively increased from almost nil to a complete 'defoliation' (99% damage) by the end of February. The studies have been supported with data in 4 tables, and illustrated with 5 figures.

INTRODUCTION

Z. rubronotata has been known since Distant's [2] description of the species from Kodaikanal, India. Mahmood [3] reported the species from Pakistan under the name *Qadria rubronotata.* The host and food plants of the species were not known untill Ahmed [1] reported it from Mirpur Khas, Pakistan as a pest of the fruit plant falsa *(Grewia asiatica).* The biology and ecology of *Z. rubronotata* has not been studied todate. The present workers recorded the species from Karachi, and studied its life history, abundance and damage caused to the host plant.

MATERIAL AND METHODS

(i) About 40 young plants of falsa were grown in pots for rearing *Z. rubronotata.* Equipment and methods used by Jabbar and Ahmed [3] for life history studies of leafhoppers were used during this study.

(ii) To study the abundance of *Z. rubronotata* during the entire period of infestation, 10 plants of falsa growing in natural conditions were carefully scrutinized at 15 days interval. At every observation, 10 heavily infested leaves on each plant were selected for making counts of adults and nymphal instars. Maximum and minimum temperatures of the day of observation were also recorded.

(iii) To determine the sex ratio in natural populations, collections were made with an aspirator at random intervals. Each sample thus collected was separately counted and the number of males and females recorded.

Fig. 1 Sleeve cage used for determining life span of leafhopper.

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†M.Sc. thesis of the first author approved by University of Karachi, Karachi.

Table 1. Fecundity of *Zygina rubronotata* (female) on falsa leaves (1977-78).

Female hatched on	Mated on	Oviposition started on	Total life (day)	Oviposited for (day)	Total eggs laid
25.X	27.X	29.X	10	6	30
31.X	3.XI	5.XI	14	9	38
31.X	2.XI	5.XI	19	14	44
28.XII	31.XII	2.I	15	10	40
31.XII	2.I	4.I	9	5	16
18.I	22.I	24.I	10	4	20
	Average		12.8	8.0	31.3

(iv) The total life span of *Z. rubronotata* was studied by releasing newly emerged adult males and females in specially designed sleeve cages (Fig. 1) on naturally growing plants in the field. The leafhoppers were observed daily in the sleeve, and the sex of those that died recorded. The experiment was continued untill all adults in the sleeve cages died.

(v) To study the longevity without feeding of *Z. rubronotata,* adult males and females, mated and unmated, newly hatched and ovipositing females, as well as newly hatched nymphal instars 1 to 5, these were caged on a paper slips in minicages (Fig. 2). These tests were replicated 10 times.

Fig. 2. Plastic minicage used for determining longevity of leafhopper without feeding.

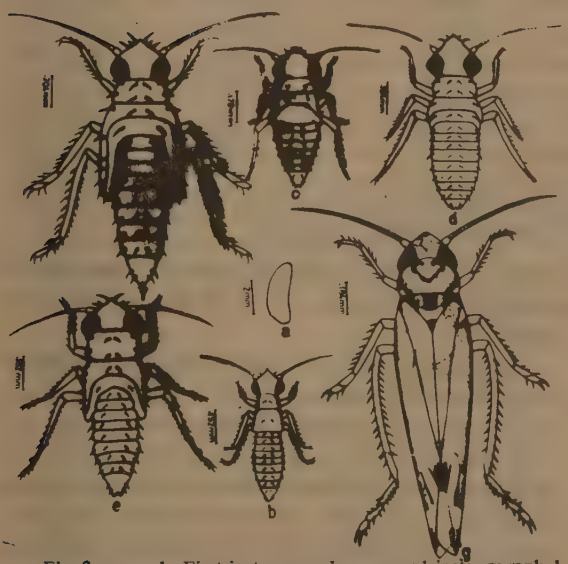

Fig. 3. a egg,b First instar nymph,c second instar nymph,d third instar nymph,e fourth instar nymph,f fifth instar nymph,g adult (male).

Table 2. Life history of *Zygina rubronotata* on falsa leaves at Karachi 1977–78.

Life history stage	Duration range (day)	Average period (day)
Egg (incubation period)	6–8	7.1
Ist instar nymph	2–4	2.8
2nd instar nymph	2–4	2.5
3rd instar nymph	2–3	2.1
4th instar nymph	1–3	1.7
5th instar nymph	1–2	1.4
Total nymphal period	8–13	10.5
Total development period	14–20	17.7
Total life span (male)	5–15	9.0
Total life span (female)	13–42	27.2

RESULTS

Life History. Studies were made on the fecundity of six mated females, life span of eleven males and eleven females, and life histories of thirty-two individual specimens of *Z. rubronotata* on falsa leaves. The results are presented in Tables 1 and 2.

Abundance of Z. rubrontata. The abundance of *Z. rubronotata* was studied at a plantation of falsa near Karachi University Campus, from early September 1977, when the leafhopper recovered from a relatively scarce occurrence during spring and summer, to late February when it became rare after a gradual decline in its population. Total catches of each of the nymphal stages as well as adults in each of the 12 samples made at 15 days interval are given in Table 3.

Table 3 shows that the number of leafhoppers was

Table 3. Total number of *Z. rubronotata* on 10 leaves of falsa with date at Karachi University Campus 1977–78.

Date	Total number of *Z. rubronotata*						
	Ist instar	IInd instar	IIIrd instar	IVth instar	Vth instar	Adult	Total population
10.IX. 77	15	4	1	–	–	–	20
25.IX. 77	66	43	20	7	5	7	148
10. X. 77	151	91	59	25	16	30	372
25. X. 77	300	164	117	30	39	64	714
9.XI. 77	434	272	161	112	71	97	1147
24.XI. 77	519	308	176	103	72	106	1284
9.XII. 77	564	372	185	113	75	122	1431
24.XII. 77	428	271	158	84	59	92	1092
8. I. 78	283	206	111	59	51	85	795
23. I. 78	200	124	96	52	34	68	574
7. II. 78	107	70	42	14	7	48	288
22. II. 78	47	25	3	2	3	15	95
Total	3114	1950	1129	601	432	734	7960

Table 4. Average damage by 'brown dust' to falsa leaves in Karachi 1977.

Dates of observation	Categories of damage and leaf (%)				
	A	B	C	Unaffected	Total affected
10.IX. 77	–	–	–	100	–
25.IX. 77	.4	–	–	99.6	.4
10.X. 77	6.8	2.1	.4	90.7	9.3
25.X. 77	13.4	8	3.2	75.4	24.6
9.XI. 77	23.4	14	8.2	54.4	45.6
24.XI. 77	36.4	18.8	11.2	33.6	66.4
9.XII. 77	25.8	25	21.4	27.8	72.2
24.XII. 77	24.2	30.2	25.6	20	80
8.I. 78	25.4	21.4	41.4	11.8	88.2
23.I. 78	11.6	15	69.8	3.6	96.4
7.II. 78	4.2	10.2	83.8	1.8	98.2
22.II. 78	2	5	92	1	99

A Leaves with damage up to 25%, B Leaves with damage 26–75%, and C Leaves with damage 76–100%.

insignificant during early September. Numbers increased rapidly and the trend of increasing population was maintained untill it reached a peak in early December. From the later half of December, the pupulation again started declining gradually. It is also apparent that the total counts of first instar nymphs were very high in the samples. Mortality in successive instars resulted in very low number of 5th instar nymphs and adults. New leaves on the plants started appearing in July and continued till the middle of September. Afterwards the leaves increased in size and the infestation increased accordingly. By the end of February the leaves were extensively damaged, almost completely covered by fungus growth and were no longer suitable for the leafhopper feeding. The leaves then soon dried and fell off.

Temperature Effect. The distribution of *Z. rubronotata* with temperature is shown in Fig. 4. The maximum counts of leafhoppers were obtained at temperature around 21° – 26° during November and December, indicating the high breeding activity of leafhopper during this period. During the later half of February and early September, when temperatures were relatively high, the leafhopper population was relatively very low and the breeding activity almost negligable. It appears that the distribution and activity of

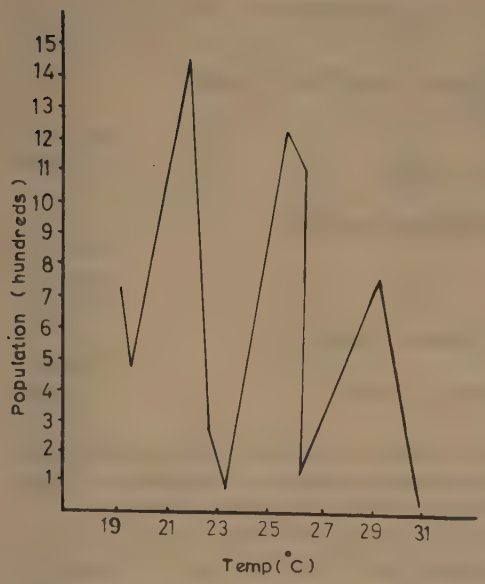

Fig. 4. Distribution of *Z. rubronotata* on falsa leaves with temperature at Karachi.

Z. rubronotata is intimately related with temperature cum winter season.

Longevity Without Feeding. Longevity without feeding of *Z. rubronotata* adults as well as its nymphal stages was checked at different temperatures and has been given in Fig. 5. Almost uniformly the survival period was high at 16^{0} and lower at higher temperature. It is obvious that when contact with the host plant is lost, the young nymphs cannot survive for long periods.

Z. rubronotata *and a Fungal Disease of Falsa.* During studies of *Z. rubronotata* on falsa, it was discovered that a fungus *Phakopsora grewiae* commonly known as 'brown dust' grew rapidly on the leaves, in the vicinity of leafhopper feeding punctures. Assessment of foliage damage by 'brown dust' was made at 15 days interval from September 1977 to February 1978. Out of 100 leaves observed each time, percentage of leaves falling into various damage categories by 'brown dust' are given in Table 4.

The 'brown dust' appeared soon after September, and steadily increased. Leaves kept free from leafhopper attack

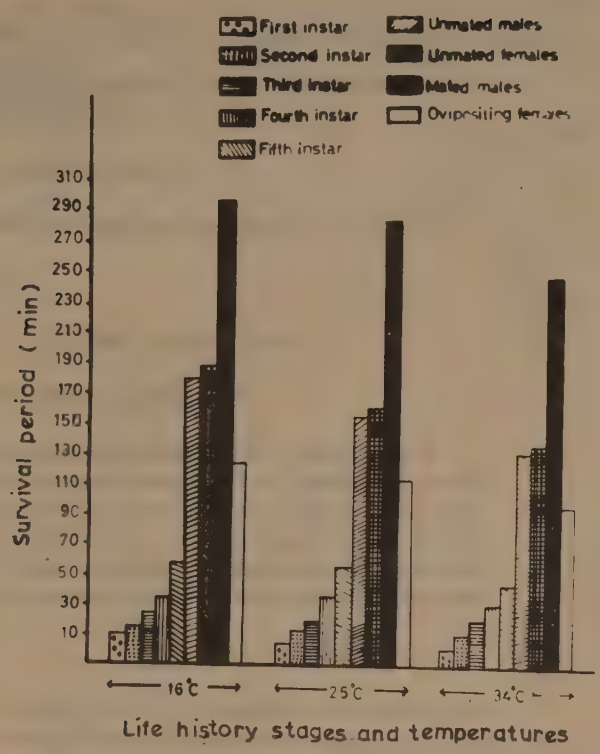

Fig. 5. Longevity without feeding of different life stages of *Z. rubronotata.*

were relatively free of 'brown dust' damage.

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DIVERSITY OF TYPHLOCYBINE LEAHOPPERS AFFECTING FRUIT PLANTS IN PAKITAN*

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A brief survey of typhlocybine leafhoppers was carried out to ascertain the species affecting fruit plants in Pakistan. Two hundered and eighty-six standardised samples were collected from different areas of the country, which showed forty-three species of leafhoppers harboured by twenty species of fruit plants. Leafhopper species turned out to be important included *Emposasca punjabensis, Typhlocyba quettensis, Typhlocyba javedi, Empoasca persicae, Erythroneura vinealis* and *Zygina rubronotata* affecting plants like apple, grape vine, peach, apricot and grewia in the country.

INTRODUCTION

As with typhlocybine leafhoppers in general, the species affecting fruit plants in Pakistan have been very little known. Ahmed [1, 2] and Samad *et al.* [3] described some typhlocybine species of fruit plants from N.W.F.P. and Baluchistan. During a recent survey, a large number of species of these leafhoppers were observed infesting the fruit plants, which appeared to be completely new to our existing knowledge of pests of fruit plants in this country. Field samples, each of which was based on insects collected in 25 sweeps of a sweep-net, from all the fruit areas of the country revealed forty-three species of typhlocybines infesting at least 20 species of fruit plants. The detailed data of various heafhopper species, affecting fruit plants in all the provinces of Pakistan has been presented in the following pages and discussed critically.

RESULTS AND DISCUSSION

The typhlocybine leafhoppers were collected mostly during summer, both in the hilly tracts of N.W.F.P., and Baluchistan, and in the plains of Punjab and Sind. Amongst the twenty species of plants on which the leafhoppers have been collected, guava, citrus, pomegranate, fig and almond appear to be relatively less or only occasionally attacked. On the other hand those observed infested by a large number of species are apple and grape vine, each infested by 14 species of leafhoppers, peach by 10 species, appricot by 7 species and walnut and cherry by 6 species each. All of these plants are almost exclusively grown in N.W.F.P., and Baluchistan.

As the leafhoppers were sampled from the fruit plants for a limited period, the diversity of leafhopper fauna harboured by them, and the abundance of various species presented in the accompanying table is not expected to be final and definitive. However, it is apparent that species like *Empoasca punjabensis, Empoasca persicae, Erythroneura rhamnicola, Typhlocyba javedi, Typhlocyba quettensis* and *Erythroneura vinealis* are most widespread and generalized in their feeding, affecting a large number of host/food plants. With regard to the mechanical damage liable to be caused to the host/food plants, the number of leafhoppers of any species would indicate the intensity of infestation. On this basis, a glance over Table 1 given above would show that reasonably heavy infestations are of *Empoasca punjabensis* on apple, mulberry and peach, of *Typhlocyba quettensis* on apple and of *Typhlocyba javedi* on peach and some others. It has also been observed that species like *Zygina rubronotata, Erythroneura vinealis,* and *Empoasca persicae* become seriously abundant on grewia (in Sind only), grape vine, and apricot respectively.

Due to discontinuous occurrence of the fruit plants in the country, it is rather premature to make a definitive comment on the natural distribution of the species harboured by them. The leafhopper species on apricot, fig and pomegranate have been collected only from Baluchistan, those of grewia from only Sind, of walnut from only N.W.F.P. and those of water melon, Zizyphus sp. and guava from only Punjab, a finding which does not preclude the distribution of their pest species in other provincial

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Table 1. Number of adult males and females of typhlocybine leafhoppers collected on fruit trees in Pakistan

Leafhopper species	Host and food plants (No. of leafhopper adults)										Total
	Almond	Cucumis	Guava	Citrus kh	Citrus kn	Mulberry	Pear	Plum	Water melon	Zizyphus	
In Punjab											
Empoasca punjabensis	–	996	8	–	–	–	–	–	1301	–	2305
Empoasca naheedi	–	–	–	–	–	–	9	–	–	–	9
Empoasca saeedi	–	–	–	–	–	–	–	1	–	–	1
Amrasca devastans	–	10	–	–	–	–	–	–	–	–	10
Austroasca khani	–	–	–	2	–	–	–	–	–	–	2
Motschulsyia sp.	–	–	1	–	–	–	–	–	–	–	1
Typhlocyba mubariki	–	–	–	–	–	3	–	–	–	–	3
Zygina bidrai	–	1	–	–	–	–	–	–	–	–	1
Zygina deraensis	–	–	–	–	6	–	–	–	–	–	6
Zygina pakistanica	9	–	–	–	–	1	–	–	–	787	797
Total No. of samples	9	42	9	1	1	2	1	1	29	1	96
Total leafhoppers	9	1007	9	2	6	4	9	1	1301	787	3135

	Grewia	Grape vine	Lemon	Mango	
In Sind					
Zygina rubronotata	130	–	–	–	130
Empoasca karachiensis	–	21	–	–	21
Austroasca vinealis	12	20	–	–	32

(Table 1 continued)

Zygina sindhensis	4	–	–	24	28
Amrasca devastans	9	57	–	–	66
Erythroneura hazarensis	–	–	12	–	12
Platyspina vinifera	–	1	–	–	1
Erythroneura vinealis	–	50	–	–	50
Total No. of samples	10	8	1	1	20
Total No. of leafhoppers	155	149	12	24	340

	Almond	Apple	Cherry	Fig	Grape vine	Peach	Pear	Plum	Walnut	Total
In N.W.F.P.										
Typhlocyba rahmani	–	–	–	–	–	–	–	–	48	48
Typhlocyba juglansae	–	–	–	–	–	–	–	–	5	5
Typhlocyba sharfi	–	–	–	6	–	–	–	–	–	5
Typhlocyba ismaili	–	1	–	–	–	–	–	–	35	36
Typhlocyba confusa	–	34	–	6	–	–	–	–	–	40
Typhlocyba ghanii	–	1	–	–	–	–	–	–	–	1
Typhlocyba quettensis	–	4	–	–	–	1	–	–	4	9
Jalalia colorata	–	–	–	–	–	5	–	–	–	5
Empoascanara ghulami	–	–	–	–	–	5	–	–	–	5

(Table 1 continued)

Empoasca persicae	7	44	–	–	–	4	4	–	4	63
Empoasca punjabensis	–	–	–	–	6	13	–	–	–	19
Erythroneura rhamnicola	10	50	–	4	5	32	3	3	7	114
E. hazarensis	–	–	–	–	4	–	–	–	–	4
E. vinealis	–	–	–	–	30	–	–	–	–	30
E. chaudhrii	–	–	–	–	8	–	–	–	–	8
E. sesamae	–	–	–	–	10	–	–	–	–	10
Zyginidia younasi	–	–	3	–	–	–	–	–	–	3
Agriahana acuta	–	2	–	–	–	–	–	–	–	2
Total samples	1	6	1	2	8	16	3	1	10	48
Total leafhoppers	17	136	3	15	63	60	7	3	103	407

	Almond	Apple	Apricot	Cherry	Fig	Grape vine	Mulberry	Peach	Plum	Pomegranate	Total
In Baluchistan											
Empoasca decedens	–	–	–	–	–	–	–	64	–	10	74
Empoasca persicae	6	509	296	57	–	52	–	368	10	–	1298
Empoasca syedi	–	–	–	–	–	–	–	–	6	–	6	
Empoasca punjabensis	2	1251	555	8	12	75	263	910	–	18	3094
Empoasca masoodi	–	8	–	–	–	–	–	–	–	–	8
Empoasca baluchi	–	13	–	–	–	5	–	38	–	–	56
Empoasca sesamae	–	–	–	–	–	–	–	3	–	–	3

(Table 1 continued)

Empoasca parastriata	–	–	13	–	–	–	–	–	–	–	13
Typhlocyba quettensis	–	1310	712	–	–	–	–	–	–	7	2029
Typhlocyba javedi	–	118	17	–	2	–	–	308	–	10	455
Typhlocyba sharfi	–	–	–	8	–	–	–	–	–	–	8
Typhlocyba sariabensis	–	59	–	–	–	–	–	–	–	–	59
Amrasca devastans	–	30	–	–	–	142	–	4	–	–	176
Amrasca elongata	–	–	–	–	–	10	–	–	–	5	15
Austroasca vinifera	–	–	20	–	–	–	–	–	–	–	20
Byphlocyta spinosa	–	70	–	150	–	–	–	–	–	–	220
Zyginidia quyumi	–	–	–	–	–	–	2	–	1	–	3
Erythroneura rhamnicola	14	50	40	3	–	–	–	350	119	–	576
Erythroneura urakensis	–	6	–	–	–	–	–	–	–	–	6
Erythroneura vinealis	–	–	–	–	–	62	–	–	–	–	62
Total samples	2	47	21	6	3	9	3	19	7	5	122
Total leaf hoppers	22	3424	1653	226	14	346	265	2045	136	50	8181

areas. By and large our important fruit plants in hilly areas of N.W.F.P. and Baluchistan appear usually heavily infested with typhlocybine leafhoppers.

As the typhlocybine leafhopper species have been observed feeding, in general on leaf tissue, or occasionally on tender skin of fruits, it is obvious that their large scale presence on the host/food plants would not only result in reduction of plant vigour, but would also cause physical damage and nutritional loss both to the fruit and leaves. The coexistence of several leafhopper species, in many cases, on the same plant also indicates lack of host plant specificity in most of these species. It is obvious on the basis of this preliminary survey that typhlocybine leafhopper complex on apple, grape vine, peach, and grewia, particularly involving species like *Empoasca punjabensis, Typhlocyba quettensis, Typhlocyba javedi, Erythroneura vinealis, Empoasca persicae,* and *Zygina rubronotata* needs thorough investigation due to its apparent serious nature. Placewise

Table 2. Summarised table showing abundance of typhlocybine leafhoppers on fruit plants in Pakistan.

Host and food plants	Punjab			Sind			N.W.F.P.			Baluchistan			Total		
	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species
Almond	9	9	1	–	–	–	1	17	2	2	22	3	12	48	3
Apple	–	–	–	–	–	–	6	136	7	47	3424	11	53	3560	14
Apricot	–	–	–	–	–	–	–	–	–	21	1653	7	21	1653	7
Cherry	–	–	–	–	–	–	1	3	1	6	226	5	7	229	6
Citrus	2	8	2	–	–	–	–	–	–	–	–	–	2	8	2
Cucumis	42	1007	3	–	–	–	–	–	–	–	–	–	42	1007	3
Fig	–	–	–	–	–	–	2	15	3	3	14	2	5	29	5
Grape vine	–	–	–	8	149	5	8	63	6	9	346	6	25	558	14
Grewia	–	–	–	10	155	4	–	–	–	–	–	–	10	155	4
Guava	9	9	2	–	–	–	–	–	–	–	–	–	9	9	2
Lemon	–	–	–	1	12	1	–	–	–	–	–	–	1	12	1
Mango	–	–	–	1	24	1	–	–	–	–	–	–	1	24	1
Mulberry	2	4	2	–	–	–	–	–	–	3	265	2	5	269	4
Peach	–	–	–	–	–	–	16	60	6	19	2045	7	35	2195	10
Pear	1	9	1	–	–	–	3	7	2	–	–	–	4	16	3
Plum	1	1	1	–	–	–	1	3	1	7	136	4	9	140	4
Pomegranate	–	–	–	–	–	–	–	–	–	5	50	4	5	50	4
Walnut	–	–	–	–	–	–	10	103	6	–	–	–	10	103	1
Water melon	29	1301	1	–	–	–	–	–	–	–	–	–	29	1301	1
Zizyphus	1	787	1	–	–	–	–	–	–	–	–	–	1	787	1
Total	96	3135	10	20	340	8	48	407	18	122	8181	20	286	12063	43

position of infestation is outlined below:

Almond. The plant grows only in the hilly tracts of N.W.F.P. and Baluchistan provinces. Of the three species of leafhoppers collected on it, *Erythroneura rhamnicola* and *Empoasca persicae* infest it in both the provinces. *E. rhamnicola* also breeds on the plant.

Apple. Apple orchards are quite common in N.W.F.P. and Baluchistan particularly in colder parts of the two provinces. The plant was observed to harbour at least fourteen species of typhlocybines, of which *Typhlocyba quettensis* and *Empoasca punjabensis* appeared most abundant and widespread. The host plant is more seriously infested in Baluchistan, where some other species like *Empoasca persicae* and *Typhlocyba javedi* were also fairly abundant. *T. quettensis* and *E. punjabensis* were observed breeding on the plant.

Apricot. The plant grows both in N.W.F.P and Baluchistan, but was observed infested by typhlocybine species in Baluchistan only. Of the seven speices collected on it, *Typhlocyba quettensis, Empoasca punjabensis* and *Empoasca persicae* were most abundant. Nymphs of *T. quettensis* were also collected along with adults.

Cherry. The plant is very sparsely grown in N.W.F.P. and Baluchistan. Of the six species of typhlocybine leafhoppers collected on the host plant, *Byphlocyta spinosa* was the most abundant, and observed breeding on the plant in Baluchistan. *Empoasca persicae* was also common in some areas on the plant.

Cucumis. The fruit plant grows all over Pakistan, particularly in warmer parts. Of the three species of typhlocybines collected, and only from Punjab, *Empoasca punjabensis* was the most dominant, and observed breeding freely on the plant.

Grape Vine. Grape vine orchards are commonly grown in N.W.F.P and Baluchistan, and to a lesser extent in Sind and Punjab. Of the fourteen species of typhlocybines collected on the plant, *Erythroneura vinealis, Empoasca punjabensis* and *Amrasca devastans* were found to be most abundant. All the three species were observed breeding on the host plant, both in N.W.F.P. and Baluchistan.

Grewia. Grewia asiatica is grown sparsely in Sind and Punjab, but the leafhopper infestation was observed only in Lower Sind. Of the four species collected from the plant, *Zygina rubronotata* was the most abundant. The leafhopper was also found responsible for spreading the fungl infection *Phakopsora grewiae* particularly in Karachi and Tandojam areas. The leafhopper freely breeds on the grewia plant from August to February, till the leaves become unsuitable for feeding of leafhoppers.

Mulberry. The plant was observed very heavily infested with *Empoasca punjabensis* in a few areas of Baluchistan only.

Peach. The plant was observed more seriously infested in Baluchistan than in N.W.F.P. Of the ten species of typhlocybines collected on it, *Empoasca punjabensis, Empoasca persicae, Erythroneura rhamicola* and *Typhlocyba javedi* were the most abundant. All the species breed on the plant throughout summer.

Plum. Of the four species of typhlocybines collected on the fruit plant, *Erythroneura rhamnicola* was observed to be the most abundant. Nymphs of the species were very rare on the plant in Baluchistan.

Walnut. Walnut plant grows in N.W.F.P. Of the six species of leafhoppers collected on it, *Typhlocyba rahmani* and *Typhlocyba ismaili* were the most abundant. No obvious damage to the host plant was observed. Both the species breed on the plant.

Water Melon. Only *Empoasca punjabensis* was observed in large numbers on the host plant in Punjab. The species also breeds on it.

Zizyphus sp. The fruit plant grows in all the provinces of Pakistan, but was observed infested by *Zygina pakistanica* in Punjab only. Casual studies confirmed the occurrence of the species in Sind. The leafhopper breeds on the plant both in Punjab and Sind.

The data in the tables above supports the contention that the fruit plants like fig, guava, citrus, mango, pear and pomegranate are not seriously affected by typhlocybine leafhoppers. On the other hand several of the forty-three leafhopper species recorded on various fruit plants, are only chance catches, and these as a routine do not attack these plants. Fruit plants of higher economic value, and affected by serious infestations of leafhoppers are concentrated in N.W.F.P., and Baluchistan.

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ABUNDANCE AND DIVERSITY OF TYPHLOCYBINE LEAFHOPPERS ON VEGETABLE PLANTS IN PAKISTAN

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A brief survey of typhlocybine leafhoppers affecting vegetable plants was made in Pakistan. The results of 1493 samples collected indicated that nearly 39 species of leafhoppers infested 37 species of vegetable plants . Of these the plants observed seriously affected were potato, pumpkin, spinach, carrot, bitter gourd, brinjal, chillies, okra, turnip, tomato and sugarbeet. Emerging out as the most important species in these studies were *Empoasca punjabensis* which affected 35 out of 37 plants species of vegetable plants.

INTRODUCTION

Vegetable plants are known to be preferred hosts of typhlocybine leafhoppers all over the world. In Pakistan however no satisfactory information was available apart from a few studies of Ahmed [1], Ahmed *et al.* [2] and Pruthi [4]. A brief survey was, therefore, conducted in the country for the first time, which showed a large number of typhlocybine species feeding on these plants. In addition to collecting as many species as could be possible, every plant species was separately sampled with the help of a hand-operated sweep net, of which 25 sweeps on a plant constituted one standard sample. Data of leafhoppers collected in 1493 such samples has been presented in the Tables 1 and 2.

RESULTS AND DISCUSSION

The data presented in the Tables indicates that most of the vegetable plants are affected by more than one, and in some cases by a large number of species of typhlocybine leafhoppers. There were e.g. 15 species on pumpkin *(Cucurbita pepo)* 12 on potato *(Solanum tuberosum)*, 10 on spinach *(Spinacia oleracea)*, 9 on carrot *(Daucus carota)*, 7 on each of bitter gourd *(Momordica charantia)*, and chillies *(Capsicum annum)*, 6 on each of tomato *(Lycopersicum esculentum)*, and brinjal *(Solanum mellongena,)* 5 on each of raddish *(Rhaphanus sativus)*, turnip *(Brassica napus)*, sugar beet *(Beta vulgaris)*, and coriander *(Coriandrum sativum)*. Vegetable plants like luffa *(Luffa cylindrica)*, okra *(Abelmoschus esculentus)*, pea *(Lathyrus odoratus)*, tobacco *(Nicotiana tabaccum)*, water gourd *(Benincasa hispida)*, white gourd *(Praecitrullus fistulosus)*, cluster bean *(Cyamopsis tetragonoloba)*, mint *(Mentha piperata)*, and sesame *(Sesamum indicum)* though not affected by a large number of species, nevertheless harboured 1–4 leafhopper species each. Vegetable plants like onion, garlic, cabbage, and cauliflower, did not appear to be suffering from any serious attack of leafhoppers, and species collected on them appeared only chance catches.

Of the 39 species of typhlocybines studied in the present survey, *Empoasca punjabensis* turned out to be the commonest and most widespread, as it was recorded all over Pakistan, and on 35 out of 39 species of vegetable plants studied. A similar situation existed with *E. punjabensis* on fruit plants in Pakistan as reported by Ahmed *et al.* [2,3]. *Amrasca devastans* is also fairly common and has been collected on 18 plants species. *Empoasca syedi* was collected on 14 species on vegetables, in N.W.F.P. only, and *Empoasca kerri* on 7 plant species in Punjab, Sind and N.W.F.P. The present survey revealed that the leafhoppers affecting vegetable plants belonged mostly to the tribe Empoascini, and were general feeders rather than host specific.

Although the total number of samples of leafhoppers collected appears fairly reasonable, but their plant-wise and region-wise number varies considerably due to the relative abundance, and varieties of vegetable plants grown in different regions of the country. It is, however, quite satisfactorily shown that most of our vegetable plants are under heavy attack of a large number of typhlocybine species. A brief discussion of important infestations is given

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Table 1a. No. of adult typhlocybine leafhoppers collected on vegetable plants in Pakistan

Leafhopper species	Host and food plants (No. of leafhoppers collected)																													
	Bauhinia	Bitter gourd	Brinjal	Cabbage	Carrot	Cauliflower	Chillies	Cholai	Coriander	Cowpea	Cucumber	French beans	Kulfa	Long cucumber	Luffa	Okra	Onion	Pea	Potato	Pumpkin	Raddish	Salad	Spinach	Tobacco	Turnip	Water gourd	White gourd	Tomato	Total
In Punjab																														
Zygina binotata	306	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	306
Empoasca punjabensis	–	2170	825	11	139	10	1143	274	432	77	541	456	26	2788	1730	222	–	50	1686	6562	123	140	1312	2680	51	1301	1470	737	26956
Empoasca kerri	–	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	30
Zyginidia quyumi	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	3
Empoasca decipiers	–	–	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30
Empoasca solanifolia	–	–	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	16
Amrasca devastans	–	–	7640	–	32	–	24	–	3	–	–	–	13	–	36	4400	30	–	53	97	30	–	10	–	–	–	–	–	–	12368
Empoasca jabbari	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14	–	–	–	–	–	–	–	–	–	14
Empoasca minor	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13	–	–	–	–	–	–	–	–	–	13
Austroasca ghaziensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	5
Total samples	4	129	82	2	22	1	38	1	21	5	40	8	2	121	64	59	12	5	82	237	21	15	59	75	9	29	32	62	1237
Total leafhoppers	306	2196	8512	11	171	10	1167	274	435	77	541	456	39	2788	1766	4622	30	55	1739	6691	153	140	1322	2681	51	1301	1470	737	39741

Table 1b

Leafhopper species	Host and food plants (Number of leafhoppers collected)																				
	Bauhinia	Bitter gourd	Brinjal	Cabbage	Carrot	Chillies	Cluster bean	Coriander	Luffa	Mint	Okra	Onion	Potato	Pumpkin	Raddish	Sesame	Spinach	Sweat potato	Tomato	Total
In Sind																				
Empoasca sindhensis	–	19	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	22
Zygina sindhensis	–	1	–	–	7	–	–	3	–	–	–	–	–	–	–	–	5	–	–	16
Amrasca devastans	–	1	108	–	18	3	–	–	–	–	118	–	72	20	–	–	–	14	70	424
Empoasca bostanensis	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Empoasca kerri	–	–	–		30	–	117	–	–	–	–	–	–	–	7	–	8	–	1	163
Empoasca mardanensis	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Empoasca punjabensis	–	–	–	–	10	–	–	–	37	6	–	1	5	5	1	182	32	–	12	291
Austroasca jamkandensis	–	–	–	–	–	7	–	–	–	–	–	–	27	–	–	–	–	–	–	34
Empoasca sesamae	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	45	–	–	–	48
Empoasca cyamopsae	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	3	–	–	–	13
Austroasca tuberosa	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2
Austroasca viniferae	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	2	–	–	4
Empoasca masoodi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	10
Empoasca syedi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12	–	–	12
Zygina binotata	240	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	240
Total samples	1	4	7	1	10	2	4	1	1	1	1	1	6	3	2	4	10	1	3	63
Total leafhoppers	240	21	108	1	78	10	130	3	37	6	118	1	106	27	8	230	69	14	83	1290

below.

Sugar Beet. It was observed largely affected by leafhoppers in Baluchistan and N.W.F.P. Of the five typhlocybine species collected, *Empoasca punjabensis* and *Empoasca syedi* were the most abundant. Both the species were recorded breeding on the plant.

Bitter Gourd. It is grown all over Pakistan, but suffered from leafhopper attack in Punjab, Sind and N.W.F.P Of the seven species collected on it, *Empoasca punjabensis* was the most abundant. To a lesser extent *Empoasca kerri* was also collected on it. *E. punjabensis* also breeds on the plant.

Brinjal. It was the most heavily infested plant by typhlocybines, almost all over the country. Of the six species recorded on it, *Amrasca devastans* was the most abundant. *Empoasca punjabensis* was also collected quite commonly on it in Punjab. *A. devastans* freely breeds on the plant.

Table 1c

Leafhopper species	Host and food plants (Number of leafhoppers collected)																				Total
	Sugar beet	Bitter gourd	Bottle gourd	Brinjal	Chillies	Coriander	Cucumber	Garlic	Kulfa	Irenu-greek	Mint	Okra	Pea	Potato	Pumpkin	Raddish	Salad	Spinach	Water gourd	Tomato	
In N.W.F.P.																					
Empoasca punjabensis	150	59	67	73	18	–	–	–	24	–	16	100	–	2	161	–	15	25	–	64	774
Empoasca syedi	191	4	–	147	1	–	15	1	–	–	–	162	–	51	224	–	16	50	3	49	914
Empoasca kerri	–	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	48
Erythroneura nagpurensis	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
Amrasca devastans	–	–	–	52	–	–	–	–	–	–	–	113	–	–	–	–	–	–	–	–	165
Zyginidia quyumi	–	–	–	–	4	–	–	–	1	–	6	–	–	–	–	–	–	–	–	–	11
Byphlocyta spinosa	–	–	–	–	1	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	4
Empoasca capsicae	–	–	–	–	7	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	9
Empoasca planata	–	–	–	–	–	84	–	–	–	–	–	–	–	–	–	–	–	–	–	–	84
Zyginidia behrinensis	–	–	–	–	–	4	–	–	–	11	–	–	1	–	–	–	–	–	–	–	16
Empoanara ghulami	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1
Erythroneura hazarensis	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	10
Empoasca khaliquei	–	–	–	–	–	–	–	–	–	–	–	47	–	18	22	6	–	–	–	–	85
Eupteryx tuberculata	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	3
Zyginidia bindrai	–	–	–	–	–	–	–	–	–	–	–	–		59	–	–	–	–	–	9	68
Empoasca apodema	–	–	–	–	–	–	–	–	–	–	–	–	–	–	18	–	–	–	–	–	18
Zygina haripurensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	2
Zyginidia sawaii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	2

(Table 1c continued)

Empoasca mardanensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20	–	–	5	–	39	64
Erythroneura vinealis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	5
Total samples	2	6	1	8	5	2	1	1	4	1	4	13	1	8	19	1	2	7	1	10			97
Total leaf-hoppers	341	113	67	272	31	88	15	1	25	11	23	432	1	128	456	6	31	80	3	161			2285

	Sugar beet	Brinjal	Carrot	Chillies	Coriander	French beans	Luffa	Fenugreek	Mint	Mustard	Okra	Pea	Potato	Pumpkin	Raddish	Spinach	Turnip	Water gourd	Tomato	Total
In Baluchistan																				
Empoasca punjabensis	1394	45	1900	1020	497	40	15	168	105	169	98	–	2003	8	1559	1798	2340	10	712	13881
Empoasca bostanensis	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	2
Empoasca persicae	–	–	137	–	–	–	–	–	–	–	–	–	149	4	–	36	–	–		326
Empoasca masoodi	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	26	–	–	32
Empoasca planata	27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	27
Typhlocyba quettensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	19	4	–	23
Zyginidia quyumi	–	–	5	–	–	–	–	–	–	–	–	–	–	–	9	4	4	–	–	22
Amrasca devastans	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	7	–	–	23
Amrasca elongata	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	6
Zygina haripurensis	9	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	–	–	15
Zyginidia sawaii	–	–	–	–	–	–	–	–	–	–	–	1	34	–	–	–	–	–	–	35
Empoasca tabaci	–	–	–	–	–	–	–	–	–	–	–	–	41	–	–	–	–	–	–	41
Total samples	10	1	8	10	4	1	3	1	2	1	1	1	4	3	8	11	16	2	9	96
Total leaf hoppers	1440	45	2042	1020	497	40	27	168	111	169	98	1	2227	14	1568	1844	2396	14	712	14433

Carrot. It was observed most heavily infested in Baluchistan, and to a lesser extent in Punjab and Sind. Of the nine typhlocybines collected, *Empoasca punjabensis* was most abundant on the plant in general. Nymphs of the species were also collected on it.

Chillies. It was observed very heavily infested by leafhoppers in Punjab and Baluchistan. Of the seven species of typhlocybines collected on chillies, *Empoasca punjabensis* was the most abundant. Occasionally specimens of *Amrasca devastans* were also recorded occuring in mixed populations with *E. punjabensis*, which also breeds on the plant.

Okra. The plant was very seriously infested by typhlocybines almost all over Pakistan. Of the five species collec-

Table 2. Summarised table of leafhopper abundance on vegetable plants in Pakistan

	Punjab			Sind			N.W.F.P.			Baluchistan			Total		
	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species	Samples collected	Total leafhoppers	No. of species
Bauhinia	4	306	1	1	240	1	–	–	–	–	–	–	5	546	1
Sugar beet	–	–	–	–	–	–	2	341	2	10	1440	4	12	1781	5
Bitter-gourd	129	2196	3	4	21	3	6	113	4	–	–	–	139	2330	7
Bottle gourd	–	–	–	–	–	–	1	67	1	–	–	–	1	67	1
Brinjal	82	8512	5	7	108	1	8	272	3	1	45	1	92	8937	6
Cabbage	2	11	1	1	1	1	–	–	–	–	–	–	3	12	2
Carrot	22	171	2	10	78	7	–	–	–	8	2042	3	40	2291	9
Cauli-flower	1	10		–	–	–	–	–	–	–	–	–	1	10	1
Chillies	38	1167	2	2	10	2	5	31	5	10	1020	1	55	2228	7
Cholai	1	274	1	–	–	–	–	–	–	–	–	–	1	274	1
Cluster-beans	–	–	–	4	130	3	–	–	–	–	–	–	4	130	3
Coriander	21	435	2	1	3	1	2	88	2	4	497	1	28	1023	5
Cowpea	5	77	1	–	–	–	–	–	–	–	–	–	5	77	1
Cucumber	40	541	1	–	–	–	1	15	1	–	–	–	41	556	2
French-beans	8	456	1	–	–	–	–	–	–	1	40	1	9	496	1
Garlic	–	–	–	–	–	–	1	1	1	–	–	–	1	1	1
Kulfa	2	39	2	–	–	–	4	25	2	–	–	–	6	64	3
Long-cucumber	121	2788	1	–	–	–	–	–	–	–	–	–	121	2788	1
Luffa	64	1766	2	1	37	1	–	–	–	3	27	3	68	1830	4
Frenugreck	–	–	–	–	–	–	1	11	1	1	168	1	2	179	2
Mint	–	–	–	1	6	1	4	23	3	2	111	2	7	140	4
Mustard	–	–	–	–	–	–	–	–	–	1	169	1	1	169	1
Okra	59	4622	2	1	118	1	13	432	5	1	98	1	74	5270	5
Onion	12	30	1	1	1	1	–	–	–	–	–	–	13	31	2
Pea	5	55	2	–	–	–	1	1	1	1	1	1	7	57	4
Potato	82	1739	2	6	106	4	8	128	6	4	2227	4	100	4200	12
Pumpkin	237	6691	5	3	27	3	19	456	9	3	14	3	262	7188	15
Raddish	21	153	2	2	8	2	1	6	1	8	1568	2	32	1735	5
Salad	15	140	1	–	–	–	2	31	2	–	–	–	11	171	2
Sesame	–	–	–	4	230	3	–	–	–	–	–	–	4	230	3
Spinach	59	1322	2	10	69	6	7	80	3	11	1844	4	87	3315	10
Sweet-potato	–	–	–	1	14	1	–	–	–	–	–	–	1	14	1
Tobacco	75	2681	2	–	–	–	–	–	–	–	–	–	75	2681	2
Turnip	9	51	1	–	–	–	–	–	–	16	2396	5	25	2447	5
Water gourd	29	1301	1	–	–	–	–	–	–	2	14	2	31	1315	3
White gourd	32	1470	1	–	–	–	1	3	1	–	–	–	33	1473	2
Tomato	62	737	1	3	83	3	10	161	4	9	712	1	84	1693	6
Total	1237	39741	10	63	1290	16	97	2285	20	96	14433	12	1495	57749	39

ted on it, *Amrasca devastans* far outnumbered any other typhlocybine. The other less abundant species was *Empoasca punjabensis*. Both *A. devastans* and *E. punjabensis* breed on the plant all over Pakistan.

Potato. Potato plants were observed seriously infested by leafhoppers in Punjab and Baluchistan, and to a lesser extent in Sind and N.W.F.P. Of the twelve species collected on it, *E. punjabensis*, and *A. devastans* were the most abundant. Both the species freely breed on the host.

Pumpkin. Pumpkin plants were extensively infested by leafhoppers in Punjab, and to a lesser extent in other provinces of the country. Of the fifteen species of typhlocybines collected on the plant, *E. punjabensis* was the most abundant, and also breeds on it. ***A. devastans*** and *E. syedi* were also recorded, but in smaller numbers on the plant.

Apart from the plants discussed above, cholai, coriander, cowpea, cucumber, French beans, kulfa, long cucumber, luffa, mint, mustard, spinach, tobacco, water gourd, and white gourd were all observed heavily infested by

E. punjabensis in Punjab, and most of them by the same species in Baluchistan. The leafhopper infestations in Sind, and N.W.F.P. remained usually at a low level during the period of study. Whereas leafhopper species like, *E. punjabensis* and *A. devastans* were the most abundant, being approximately 70% and 22% respectively of the total typhlocybine catches on vegetable plants. The species like *Zyginidia quyumi, Austroasca ghaziensis,* in Punjab, *Empoasca bostanensis, Empoasca mardanensis, Austroasca tuberosa,* and *Austroasca viniferae* in Sind, *Byphlocyta spinosa, Empoanara ghylami, Eupteryx tuberculata, Zygina haripurensis, Zyginidia sawaii,* and *Erythroneura vinealis* in N.W.F.P. and *Empoasca bostanensis,* and *Amrasca elongata* in Baluchistan appeared to be only chance captures on vegetable plants.

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CHLOROPHYLL LOSS IN LEAVES OF SOME PLANTS CAUSED BY LEAFHOPPER FEEDING*

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In order to determine the effect of leafhopper feeding and chlorophyll loss, studies were made on three different species, two typhlocybines and one idiocerine feeding on bauhinia, grewia and mango respectively. The chlorophyll loss turned out to be as high as 71% from the leaves, resulting into almost functional death of leaves. It was also assessed that the chlorophyll loss in leaves was proportional to the number of leafhoppers feeding. In case of typhlocybines the leaf areas affected were visible by white stippling marks, whereas in case of idiocerine leafhopper the stippling marks are not visible, but the chlorophyll is lost as usual during leafhopper feeding.

INTRODUCTION

It is fairly well-known that various species of leafhoppers, even in related genera, feed on different tissues of mainly leaves of their host/food plants [12]. Apart from rendering other losses, one of the direct results of leafhopper feeding is the chlorophyll loss in leaves. Smith [14] pointed out that chlorophyll loss in cells of the mesophyll was due to the salivary toxins injected by leafhoppers before feeding. Kennedy [7] concluded that deficiencies in chlorophyll in leaves can bring about limitations in photosynthesis, and thus affect the healthy growth of plants. Emerson [5] was of the opinion that there existed a direct relationship between the chlorophyll content and the amount of food formed. Poos and Johnson [11] discussed the role of leafhoppers in this context and reported that plant growth was inversely proportional to the number of leafhoppers feeding on it.

From the studies referred above it appeared that chlorophyll being a constituent of high biological significance in plant tissues, the role of leafhoppers in depriving the leaves from it could also bear important consequences. Whereas the various causes of chlorosis in plants and its effects have been sufficiently investigated, the extent of chlorophyll loss brought about by leafhoppers has not been studied. Nothing was known on this aspect of leafhopper biology so far in Pakistan. The present work was done to explore the extent of chlorophyll depletion by three different types of leafhoppers, an empoascan, an erythroneurine and an idiocerine feeding on three species of plants, i.e. grewia, bauhinia and mango respectively in Karachi, Pakistan.

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MATERIAL AND METHOD

1. In case of *Bauhinia variegata,* and *Grewia asiatica* affected by feeding of leafhoppers *Zygina binotata* and *Austroasca* sp. respectively five leaves of equal sizes and same age, but with different levels of infestation were picked up for estimation of their chlorophyll content. Of the five leaves picked, one was completely free from infestation, and the other four with varying number of leafhoppers (both adults and nymphs) feeding on them. The leaves of mango were of three types, all covered with sleeves, of which one was kept completely free of leafhoppers, and the other two experimentally infested with 30 and 60 leafhoppers respectively, which were allowed to feed for a period of 40 days after which leaves were also removed for comparative chlorophyll estimation.

2. The leaves were crushed separately and their homogenates prepared in acetone. The optical density in each case was plotted on a spectrophotometer, and the estimation of chlorophyll made following the method of Maclachlan and Zalik [8]. The results have been presented in the Table 1.

DISCUSSION

The leafhopper species, *Zygina binotata* as stated by Naheed *et al.* [9] feed on the undersurface of leaves, and normally do not attack any other part of the plants *Bauhinia variegata.* Like all other mesophyll feeders, the signs of leafhopper feeding appear as 'stippling' marks or rounded whitish spots on the upper surface of leaves. The leafhop-

Table 1. Chlorophyll loss in plant leaves due to feeding of leafhoppers.

Treatment No.	Average No. of leafhopper/leaf	Average stippling mark/leaf	Average chlorophyll content (mg/g)	Average loss of chlorophyll (mg/g)	Loss (%)	S.E.
In Bauhinia (by *Zygina binotata*)						
1	Nil	Nil	1.6387	–	–	±–
2	15.63	368	1.5146	0.1241	7.73	±0.94
3	23.00	720	1.4504	0.1883	11.49	±1.3
4	30.55	1177	0.9488	0.6899	42.10	±0.67
5	36.75	2062	0.4709	1.1678	71.26	±2.76
In Grewia (by *Austroasca* sp.)						
1	–	–	1.8523	–	–	–
2	15.36	375.0	1.3727	0.4796	25.0	±2.08
3	23.12	806.6	1.0112	0.8411	45.41	±1.04
4	31.17	1296.0	0.9633	0.8890	47.99	±2.89
5	33.42	1707.6	0.8834	0.9689	52.31	±2.48
In Mango (by *Idiocerus clypealis*)						
1	Nil	–	1.5279	–	–	–
2	30	–	1.0745	0.4534	29.7	±1
3	60	–	1.0297	0.4982	32.5	±1

*For each treatment averages were drawn from 3 replicates.

pers usually heavily infest the plant during their peak periods. Table 1 indicates that the amount of chlorophyll destroyed is directly proportional to the number of leafhoppers feeding.

In case of *Bauhinia variegata,* the loss of chlorophyll ranged from 7.73 to 71.26% ($\bar{X}$ 33.145), when the average leafhoppers were 26.43. As the individual plants had been observed affected by a much heavier population, the loss of chlorophyll could be still heavier. As pointed out by Naheed *et al.* [9] in such severe infestations the entire leaf gets deprived of all of its chlorophyll content, dries up and falls off.

The feeding mechanism, symptoms, and nature of damage to the plant falsa, *Grewia asiatica* by *Austroasca* sp. is similar to one caused by *Zygina binotata* to *Bauhinia variegata.* The infestation of 15.36 to 33.42 leafhoppers per leaf resulted into chlorophyll loss of 25.9 to 52.31%. It has been observed that the new leaves which appear in March–April, soon get infested by the leafhoppers, and loose their greenish appearance due to the feeding of *Austroasca* sp. In July–August, another leafhopper species *Zygina rubronotata,* which is relatively rare from March to July, starts infesting the plant, rapidly accompanied by a large-scale fungal infection. The plant suffers from heavy attack of leafhoppers almost throughout the year.

Idiocerus clypealis is known to feed on phloem tissue of mango leaves, and so the nature of damage is not identical to that of the two species described above. No 'stippling' marks appear, and so the damage symptoms are not easily recognised. The leafhoppers are found on the plant almost throughout the year, but a serious infestation is observed from beginning of April to end of October, in Karachi area. Apart from depriving the plant of its nutrients, and reducing the vigour of the plant, the leafhopper also reduces the chlorophyll content from leaves up to 32.5%, when 60 leafhoppers per leaf are allowed to feed for 40 days.

Several workers all over the world have studied the relationship between chlorophyll loss due to leafhopper feeding and various aspects of plant health. Smith [14] specifically related that white stippling marks, that usually appear on the upper surface of leaves due to feeding of typhlocybine leafhoppers are the result of destruction of chlorophyll in mesophyll cells. Saxena [13] stated that the enzyme chlorophyllase in *Empoasca kerri,* and *Amrasca devastans* acts upon the chlorophyll of leaf tissue to yield diffusable substances. Putman [12] reported that in another typhlocybine species *Typhlocyba pomaria,* the

contents of affected cells quickly break down into a homogenous mass, from which chloroplasts disappear and chlorophyll is bleached away. Poos and Johnson [11], Delong [4], and Wilson [15] all reported that loss of chlorophyll was closely related with leafhopper feeding. Jayaraj [6] described that surface area of root and stem in castor plant are greatly reduced due to feeding of leafhoppers. Peinkowski and Medler [10] reported that loss of chlorophyll in alfalfa is proportional to the number of leafhoppers present. In spite of several studies on the significance of chlorophyll loss due to leafhopper feeding, estimates of loss percentage brought about by leafhoppers was not studied so far. The present work had demonstrated that the loss could be high and may have serious consequences, as alongwith chlorophyll, other nutrients, which are so important for the quantity and quality of developing fruit on the plant, are also lost in the process of leafhopper feeding. In a few studies, made in Pakistan, on this aspect of leafhopper ecology, e.g. the quantitative losses to yield have been stated to be fairly high [2]. Ahmed *et al.* [3] studied the effects of insecticidal control of leafhoppers of potato on the quantity and quality of potato tubers and concluded that not only yield, but the nutritional value of potato tubers is also seriously affected by leafhopper feeding. Ahmed [1] observed that *Idiocerus clypealis* feeding on mango plants reduced the growth rate of young plants, by affecting the rate of appearance of new leaves, slowing down the development of length and breadth of leaves, and finally limiting the overall height of young plants. It would be worthwhile if the ultimate impact of chlorophyll loss is studied on the quantity, and quality of fruit of those host plants which are known to be heavily infested by leafhoppers, e.g. grape vines and apple in N.W.F.P. and Baluchistan, potato, tomato, tobacco, and several other plants species all over the country.

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Short Communication

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MARINE FISH NEMATODES OF PAKISTAN

Part XI. Occurrence of *Indocucullanus longispiculum* diacanthi in the Fish *Arius serratus* (Day) of Karachi Coast

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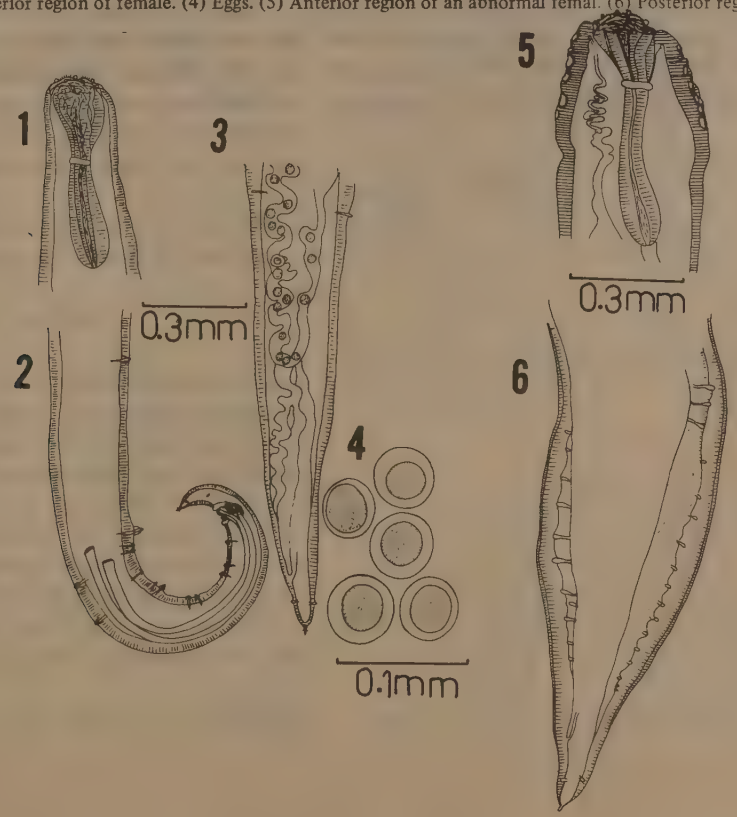
Among nematode parasites, great morphological variations exist within the species and sometimes it is difficult to point out definitely whether certain character differences in a population of nematodes represent variations or separate species. No doubt there are certain internationally approved rules for the classification of nematodes but no consideration for variations is included in such classifications. Sometimes, two widely separated species are reported as the same species considering that the host is same and the genus is same. On the other hand one species by two different authors from two different localities and hosts is reported as two separate species. The author had discussed these points while reporting some trematodes of fishes [3,4].

At present the occurrence of *Indocucullanus longispiculum diacanthi* [2] is reported from the fish *Arius serratus* of Karachi coast and the validity of this subspecies is justified. This nematode was originally described from the fish *Pseudosciaena diacanthus* [2] from which closely related specimens *Indocucullanus longispiculum* [1] were reported by Khan [1]. The present findings indicate that constant occurrence of same morphological variations in a species, and occurrence of the same in different hosts can be used to justify the validity of a particular subspecies.

MATERIALS AND METHODS

Eleven specimens of the fish *Arius serratus*, from the Karachi coast, were examined for nematode parasites during 1977. At three different occasions 3 fishes were found to harbour nematodes of the genus *Indocucullanus*. A total of 5 male, and 31 female specimens were recovered from the stomach and intestine of these hosts. The specimens were fixed in 70% alcohol and cleared in a mixture

Fig. 1–6. *Indocucullanus longisipiculum diacanthi* Bilqees *et al.* 1977 from the fish *Arius serratus:* (1) Anterior region of male. (2) Posterior region of male. (3) Posterior region of female. (4) Eggs. (5) Anterior region of an abnormal femal. (6) Posterior region of the same.



of equal quantity of 70% alcohol and glycerine. For a closer examination temporary mounts were prepared in glycerine. Diagrams were prepared with a camera lucida. Specimens are kept in the School of Parasitology, Department of Zoology, University of Karachi (Male specimen Nos. SPUK 2511 – SPUK 2518. Female specimen Nos. SPUK 2500–SPUK 2510).

OBSERVATIONS AND DISCUSSION

Indocucullanus longispiculum diacanthi Bilqees et al. (Figs. 1–6)

Measurements of the Specimens

Male. Body size, 3.10–3.28 by 0.18–0.26 mm. Esophagus length, 0.45–0.57 mm. Pseudobuccal capsule, 0.14–0.18 by 0.14–0.15 mm. Distance of nerve ring from the anterior end, 0.19–0.26 mm. Right spicule, 0.84–1.01 mm in length and the left, 0.91–1.11 mm in length; caudal papillae, 10–14 pairs including 6–9 pairs preanal, 3–4 pairs postanal, and 1 pair adanal. Tail length, 0.10–0.13 mm.

Female. Body size, 2.38–3.4 by 0.3–0.6 mm. Pseudobuccal capsule, 0.15–0.18 by 0.14–0.16 mm. Esophagus length, 0.48–0.58 mm. Distance of nerve ring from the anterior end, 0.19–0.22 mm. Distance of vulva from the anterior end 1.44–1.75 mm. Ova 0.039–0.050 by 0.03–0.05 mm. Tail length, 0.13–0.15 mm.

The species *Indocucullanus longispiculum* [1] has been reported from the fish *Pseudosciaena diacanthus* (Lac.) of Karachi coast. Recently Bilqees *et al.* [1] recovered similar nematodes from the same fish host and locality but designated these specimens as a subspecies of the species and named *I. longispiculum diacanthi* on the basis of considerable morphological variations between the specimens recovered by Khan [1] and Bilqees *et al.* [1].

It was desirable to find out if this subspecies occurs in host other than *Pseudosciaena diacanthus.* After a continuous examination of many species of fishes for several months, specimens similar to the subspecies were recovered from the fish *Arius serratus.* A closer examination revealed that these were *I. longispiculum dicanthi.* The repeated recovery of the subspecies from a different fish host suggests that it is a valid subspecies.

Some abnormalities were noticed in one female specimen which the author thinks worth reporting. This female specimen has 6 pairs of chitinous plates arranged in a single row at the anterior end of the body, 3 pairs on each side, embeded in the cuticle. Similarly at the posterior end in addition to the usual one pair of postanal papillae there were numerous preanal papillae (17 papillae at one side and 11 papillae at the other side).

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Technology Section

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TALC-CHLORITE ROCKS IN ATTOCK SLATES

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Dark grey to whitish rocks from Attock Khurd, previously described as low grade soapstone, were found to contain talc with a subordinate amount of chlorite (penninite). Other impurities are quartz, calcite, dolomite, magnesite and pyrite. The mineral assemblage has in all probability resulted from magnesium-containing solution of hydrothermal origin acting on illite of the slate.

INTRODUCTION

Near Peshawar a large outcrop of dark slates occur from Khyber Agency to Attock. The extrapeninsular representative of the Vindhyans are thought to be largely present in the belt of unfossiliferous sedimentary rocks that lies between the crystalline rocks of the central and the younger rocks of the outer Himalayas [1]. The Attock slates are thus considered to be of Cambrian age. However, Taherkheli [2] believes that they are of Jurassic age. Few, limestones and sandstones deposits are also enclosed in the slates. The slates are also permeated with trap intrusions.

At Kund (Fig. 1) near Attock Khurd small scale mining of dark grey to whitish soapstone has been continuing for years [3]. Formerly the material was used locally for soap-making and white-washing. At present it is consumed by the porcelain tile factory and as fillers in insecticide industries.

The present investigation was conducted in order to ascertain the mineralogy and chemistry of the materials.

OCCURRENCE

The main deposits of talc–chlorite are located near the Kund village (area ABCD, Fig. 1) as lenticular masses within the pelitic rocks. Some pits have been also opened up at about 800 m north of the village Kati Miana, in the strike direction of Kund deposits. The strike of the rocks is almost East–West. Fresh slates are dark in colour and contain oxidized iron pyrites. Arenaceous and calcareous bands are also found within the slates. The limestones are sometimes dolomitic. Dolerite dykes are quite frequent.

The following samples were obtained from the area for the investigation:

K1, soapy slate, black but soft, collected from the railway cutting about 20 m north of the railway crossing.

K2, highly weathered calcareous soapy slate from a zone about 60 cm thick, collected from the locality as above.

K3, slates with pyrite near K2.

K4, black slate adjacent to the sample K3.

K5 soft soapy rock with iron pyrite crystals, greyish-black saturated with water when fresh, becomes whitish grey with greenish sheen on drying, collected from one of the pits on the left side of the track.

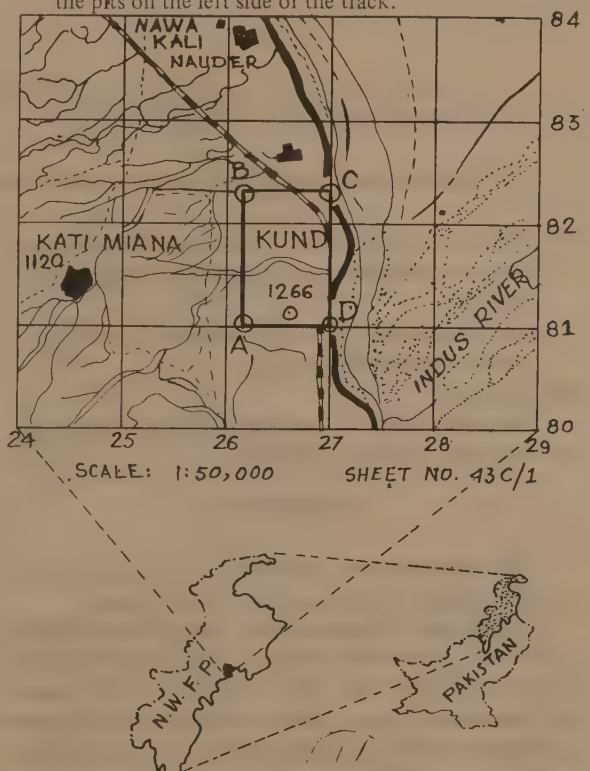

Fig. 1. Location map of talc-chlorite rocks in Attack slates.

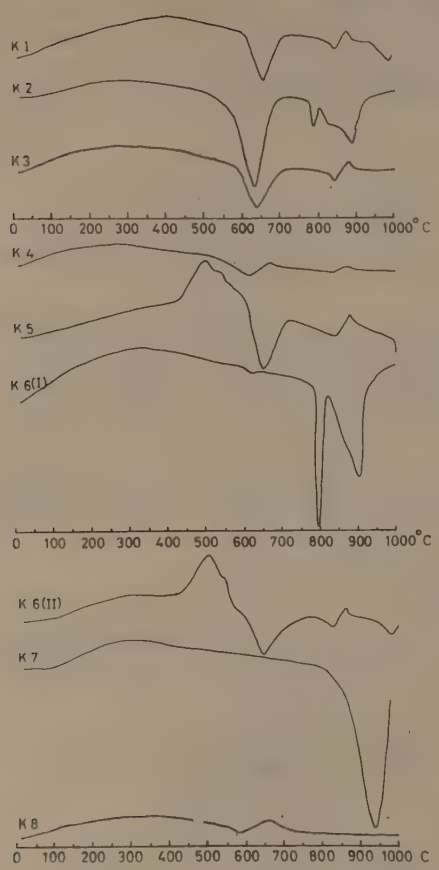

Fig. 2. Differenlial thermal curves of talc-chlorite rocks in Attock slates.

K6, soft greyish friable, soapy rock, with pure white mineralized patches, specks of iron pyrites visible, location near K5.

K7, black limestone, 61 m thick interbedded with slates, pyritic, about 440 m from K5.

K8, slate near K7.

The slate sample K6 was separated into a white fraction K6(I) and grey fraction K6(II).

MINERALOGY

The samples were examined by differential thermal analysis, X-ray diffraction and chemical techniques.

Differential Thermal Analysis. The differential thermal curves (Fig. 2) for samples K1, K2, K3, K4, K5 and K6(II) show the characteristic reaction for chlorite. The sample K2 also contains the endotherms of dolomite and the samples K5 and K6(II) have marked similarity with exothermic peaks at about 500°6 which may be due to the decomposition of FeS_2 to FeS + S and oxidation to sulphate. Samples K6(I), K7 and K8 contain dolomite, calcite and illite

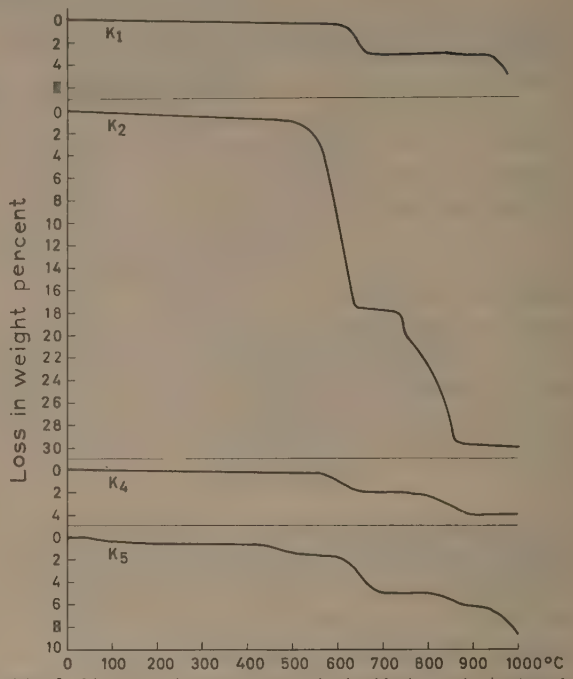

Fig. 3. Thermsgravimetric curves of talc-chlorite rocks in Attock slates.

respectively.

Thermoqravimetric Analysis. Figure 3 reproduce the thermogravimetric curves for samples K1, K2, K4 and K5. The loss due to chlorite is prominent in all the curves at about 600°.

X-Ray Diffraction. X-ray analysis were made on all the untreated samples (Table 1). Some of the samples have been examined after acid (dil HCl for 2 hr at 80°) and ethylene glycol treatments. Absence of chlorite reflections in acid-treated samples confirms that the chlorite has been decomposed. All the samples remained unaffected by the glycol treatment.

From the X-ray powder data the following conclusions regarding the mineralogy may be made (three main reflections of standard minerals from ASTM Index are given in the bracket).

K1, talc (9.34, 3.12, 4.66) with subordinate amount of chlorite (7.19, 4.80, 3.60).

K2, talc, chlorite, magnesite (2.74, 2.10, 1.70), calcite (3.04, 2.29, 2.10) and dolomite (2.89, 2.19, 1.79).

K3, quartz (3.34, 4.26, 1.82), chlorite and calcite.

K4, quartz, chlorite and illite (10.0, 4.48, 3.33).

K5, talc and chlorite.

K6(I), talc and dolomite.

K6(II), talc and chlorite.

K7, calcite.

K8, quartz, chlorite, illite and pyrite (1.63 2.71, 2.42).

Chemical Analysis. Chemical composition data for sam-

Table 1. X-ray diffraction powder data (Debye-Scherrer camera 114 mm dia; radiation (CuK) 1.54 Å exposure time 6 hr; (35 kV, 18mA).

K1		K2		K3		K4		K5		K6(i)		K6(ii)		K7		K8	
d(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I	*d*(Å)	I
–	–	–	–	–	–	–	–	13.59	20	–	–	14.48	70	–	–	–	–
9.30	90	9.30	25	–	–	9.32	2	9.50	25	9.35	50	9.30	80	–	–	9.82	2
7.19	10	7.08	20	7.08	10	7.08	5	7.08	10	–	–	7.13	70	–	–	7.08	10
–	–	4.69	20	4.79	5	4.79	2	4.79	15	4.73	60	–	–	–	–	–	–
4.57	80	4.61	1	–	–	4.51	5	4.55	5	4.59	10	4.57	30	–	–	4.43	2
–	–	–	–	4.27	15	4.24	20	–	–	–	–	–	–	–	–	4.25	25
–	–	–	–	4.04	2	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.78	5	–	–
3.57	10	3.56	30	3.56	25	3.53	2	3.56	35	–	–	3.56	60	–	–	3.53	1
–	–	–	–	3.36	100	3.36	100	3.30	1	–	–	–	–	3.35	15	3.35	100
–	–	3.26	1	3.20	5	–	–	–	–	–	–	–	–	–	–	3.20	1
3.13	80	3.13	40	–	–	3.18	20	3.13	50	3.11	70	3.12	100	–	–	–	–
–	–	3.02	40	3.02	3	3.00	2	–	–	–	–	–	–	3.02	100	3.02	1
–	–	2.88	25	2.81	1	–	–	2.84	1	2.88	100	2.84	5	–	–	–	–
–	–	–	–	–	–	–	–	2.71	1	–	–	–	–	–	–	–	–
2.60	5	2.57	50	2.57	1	2.58	20	2.59	1	–	–	2.59	30	–	–	2.59	10
–	–	–	–	2.54	1	–	–	2.54	3	–	–	–	–	–	–	–	–
2.48	15	2.49	5	2.45	5	2.46	8	2.49	1	2.48	20	2.46	30	2.49	15	2.46	10
–	–	–	–	2.39	1	2.39	4	2.39	1	2.42	5	–	–	–	–	2.39	1
2.23	5	2.28	1	2.28	3	2.28	1	2.25	1	–	–	2.25	5	2.28	25	2.29	10
–	–	2.18	5	–	–	–	–	–	–	2.19	30	–	–	–	–	–	–
–	–	2.11	5	2.13	1	–	–	–	–	–	–	2.10	25	–	–	2.01	10
2.01	10	2.01	5	2.00	1	1.96	20	2.00	10	2.01	20	2.01	50	2.09	30	–	–
–	–	–	–	–	–	–	–	1.87	1	–	–	1.87	10	1.87	30	–	–
–	–	–	–	1.82	10	1.81	25	1.83	1	1.79	30	1.82	10	–	–	1.82	15
–	–	1.71	25	–	–	–	–	1.63	1	–	–	–	–	1.60	1	1.66	20
–	–	–	–	–	–	–	–	1.56	2	–	–	1.56	15	–	–	–	–
1.53	60	–	–	1.54	10	1.54	12	1.54	5	1.52	8	1.53	40	–	–	1.54	12
–	–	–	–	–	–	1.50	1	1.50	3	1.46	8	1.50	15	–	–	–	–
–	–	–	–	–	–	–	–	–	–	1.43	5	–	–	1.44	2	–	–
–	–	–	–	1.37	5	1.37	5	1.39	4	1.38	10	1.39	40	1.42	1	1.37	1
–	–	–	–	–	–	–		1.32	1	–	–	1.31	7	–	–	–	–
–	–	–	–	–	–	–	–	1.29	1	–	–	1.29	7	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.20	1
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.18	1

Table 2. Chemical analyses

Chemical Composition	K1 (%)	K2 (%)	K4 (%)	K5 (%)
SiO_2	48.44	20.20	60.40	40.90
Al_2O_3	12.08	4.60	21.30	12.63
Fe_2O_3	0.40	2.03	0.08	3.19
FeO	1.65	1.93	3.51	2.15
MnO	Nil	Trace	Trace	–
TiO_2	0.25	0.22	0.43	0.30
CaO	0.13	9.02	0.51	0.21
MgO	29.21	32.01	5.92	28.19
Na_2O	Nil	Nil	1.52	–
K_2O	Nil	Nil	1.37	–
P_2O_5	Nil	Trace	Trace	–
H_2O^-	0.11	0.13	0.08	0.27
H_2O^+	7.64	2.28	4.04	10.96
CO_2	Nil	28.07	Nil	–
SO_3	0.42	Nil	0.51	–
S	–	–	–	2.31
Total:	100.33	100.49	99.79	101.11
Less O=S				0.82
				100.29

Table 3. Calculated mineral composition.

	K1	K2	K4	K5
$Mg_6Si_8O_{20}(OH)_4$	62.37	26.68	–	51.86
$Fe_{12}Si_8O_{20}(OH)_{16}$	4.17	1.56	6.10	1.70
$(Mg,Al)_{12}Si_8O_{20}(OH)_{16}$	30.54	11.63	19.09	31.86
$K_2Al_4(Si_6Al_2)O_{20}(OH)_4$	–	–	11.54	–
$Na_2Al_4(Si_6Al_2)O_{20}(OH)_4$	–	–	18.79	–
SiO_2 (quartz)	–	–	39.91	–
$CaCO_3$	–	8.78	–	–
$MgCO_3$	–	29.05	–	–
$FeCO_3$	–	3.10	–	–
$CaCO_3.MgCO_3$	–	16.26	–	–
$CaSO_4$	0.31	–	0.85	–
$MgSO_4$	0.35	–	–	–
FeS	–	–	–	6.35
Excess SiO_2	0.81	–	–	–
Excess Al_2O_3	–	–	1.78	–
Excess MgO	–	1.68	–	1.82
Excess CaO	–	–	0.15	0.21
Excess TiO_2	0.25	0.22	0.43	0.30
Excess H_2O	0.11	0.13	0.08	3.72
Total	98.91	99.09	98.72	97.82

ples K1, K2, K4 and K5 are given in Table 2 and calculated norms in Table 3.

The calculated composition have been based on information available from X-ray and TGA, and on the assumption that sulphur is present as FeS, sulphate as $CaSO_4$ and $MgSO_4$, CO_2 as $CaCO_3$, $MgCO_3$ $CaCO_3$, $MgCO_3$ and $FeCO_3$. Titania is assumed to be present as rutile or anatase.

The iron left after calculating $FeCO_3$ (if any) was assigned to $Fe_{12}Si_8O_{20}(OH)_{16}$. Soda and potash were assigned to $Na_2Al_4(Si_6Al_2)O_{20}(OH)_4$ and $K_2Al_4(Si_6Al_2)O_{20}(OH)_4$ respectively. After calculating paragonite and muscovite the alumina left over was assigned to form penninite $[(Mg_8Al_4)(Si_4Al_4)O_{20}(OH)_{16}]$. The magnesia left at this stage was assigned to talc $[Mg_6Si_8O_{20}(OH)_4]$.

Table 4 clearly shows that chlorite is present in all the four samples and varies between 13.19 – 34.71%. The samples K1, K2 and K5 contain talc between 26.68 – 62.37%. The slate sample K4 does not contain talc but contains about 39.91% free quartz.

The sample K2 is rather unique; it contains not only talc and chlorite but also calcite (8.78%), magnesite (29.05), siderite (3.10%) and dolomite (16.26%).

ORIGIN

The parent material for the formation of the mineral assemblage seems to be the greyish-black slate containing quartz and mica mineral (illite). The nearby slates are permeated by basic igneous rocks. The slates near the mineralized zone contain some chlorite and are free from talc. The mineralized zone contains talc, chlorite, sometimes dolomite, magnesite and also calcite.

The chlorite group mineral has been accepted to be derived by the aggradation of less organized sheet mineral celadonite, by the degradation of preexisting ferrogmagnesian minerals, and by crystallization from dilute solution of their components [4].

Chlorite group minerals are common constituents of Attock slates – from Khyber to Attock [5]. However, talc is the abundant fraction of the mineralized zone with chlorite in subordinate amount. It seems that magnesia containing hydrothermal solution must hve converted some of the muscovite and paragonite into chlorite, as in sample K4, with the following probable reaction:

$$2K_2Al_4(Si_6Al_2)O_{20}(OH)_4 + 8MgO + 6H_2O \longrightarrow$$

Muscovite

$$(Mg_8Al_4)(Si_4Al_4)O_{20}(OH)_{16} + 8SiO_2 +$$

Chlorite Quartz

$$4KOH + 2Al_2O_3$$

Removed in solution

However, under certain condition all the mica minerals

were assimilated with the formation of talc and chlorite as in samples K1, K2 and K5, with the following reaction:

$$(Mg_8Al_4)(Si_4Al_4)O_{20}(OH)_{16} + 4SiO_2 \longrightarrow$$

$$Mg_6Si_8O_{20}(OH)_4 + 2MgO + 4Al_2O_3 + 6H_2O$$

Talc — Removed in solution

At lower temperatures chlorite may be converted to talc by CO_2 metasomatism [6] and in presence of excess of CaO and CO_2 talc is unstable. As the sample K2 contains talc, chlorite, dolomite, calcite, magnesite and siderite, the reactions may be expressed as follows:

$$(Mg_8Al_4)(Si_4Al_4)O_{20}OH)_{16} + 2CO_2 + 4SiO_2 + O_2 \longrightarrow$$

$$Mg_6Si_8O_{20}(OH)_4 + 2MgCO_3 + 4Al_2O_3 + 8H_2O$$

Removed in solution

$$Mg_6Si_8O_{20}(OH)_4 + 6CO_4 \longrightarrow$$

$$6MgCO_3 + 8SiO_2 + 2H_2O + O_2$$

$$Mg_6Si_8O_{20}(OH)_4 + 6CaO + 12CO_2 \longrightarrow$$

$$6CaCO_3.MgCO_3 + 8SiO_2 + 12H_2O$$

$$Fe_{12}Si_8O_{20}(OH)_{16} + 12CO_2 \longrightarrow$$

$$12FeCO_3 + 8SiO_2 + 8H_2O$$

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EFFECTS OF HERBICIDES ON THE RATE OF GERMINATION AND RESPIRATION OF COTTON SEEDS

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Delinted seeds of *Gossypium hirsutum* L. cv. M-100 were treated with prometryne, cotoron and their mixture (1:1). Percentage germination and rate of respiration were recorded after regular intervals.

A highly significant increase in the rate of respiration was found to be associated with a markedly reduced percentage of germination as a result of cotoron application. Results obtained from the mixture were significant at $P < .05$, whereas the effect of prometryne was insignificant on either parameters.

INTRODUCTION

Varied responses of plants to different herbicides under various conditions provokes an extensive toxicological study to evaluate the suitability of various herbicide species to Pakistani crops.

In general, triazines have been reported to cause irreversible mitotic disturbances [1] and inhibition of respiration associated with oxidative phosphorylation [2]. Substituted urea compounds are, however, considered to be responsible for acceleration of respiration [3], swelling and distortion of mitochondrial [4] and retardation of mitosis in various tissues [5].

For weed control in cotton, prometryne (6-methylmercapto-2-4-bis(isopropylamino)-s-triazine) and cotoron (3-trifluoromethyl-phenyl)-1, 1-diethyl urea) are generally recommended at a rate of 3–4 kg/ha as pre- or postemergence measures.

The present investigation is on the germination and respiratory responses of the seeds of a widely cultivated variety of cotton M-100 to promotryne, cotoron and their equal mixture.

EXPERIMENTAL

Germination trials were expedited on Whatman filter paper No.1 in 10-cm dia petri dishes each containing ten acid delinted seeds of *Gossypium hirsutum* L.cv. M-100. Prometryne, cotoron and a mixture (1:1) of both were applied at a rate of 440 ppm (approx. 3 kg/ha) using 5 ml aqueous solution per petri dish. Four replicates were maintained for each treatment and control in dark at 32° and 27° for 12-hr alternate duration. Distilled water was used for successive watering and the rate of germination was recorded daily.

Warburg's constant volume respirometer was employed to entail the respiratory behaviour of the seeds [6]. The main chamber of the Warburg's flask contained 2 ml phosphate buffer (pH 6.8) and the seeds. Whereas the central well was loaded with 0.5 ml of 10% KOH. The herbicide doses were mixed with the buffer to attain 440 ppm concentration. Six replicates of five seeds each were maintained for every treatment.

About 7 hr after administering the herbicides, the flasks were shaken at 30 strokes/min in dark at 27°. From the hour 8 onwards the manometric levels were monitored after every 10 min.

RESULTS AND DISCUSSION

It is apparent from Table 1A that both the herbicides and their equal mixture reduce the germination percentage.

As compared to the control, the rate of germination on the second day of the experiment was 52.37% for prometryne, 28.57% for cotoron and 47.62% for the mixture-treated seeds. On the third day the same ratios were 40.63, 34.38 and 31.25% for the treatments of prometryne, cotoron and the mixture, respectively.

The controls attained their maximum on the 4th day by exhibiting 85% germination, whereas the seeds treated with prometryne were able to cover 52.94% and those with cotoron and the mixture up to 38.24% of the control.

Germination was abated in the cotoron-treated seeds from the 4th day onward. Nevertheless, germination rate on the 5th day crept up to 73.53% near the control in the prometryne-treated seeds. Those treated with the mixture could follow up to 44.12%. The final ratio of treatments to control was 76.74% for prometryne and 55.88% for the mixture. No further seed germination was observed in any of the replicates on the 7th day. Final observations tabulated for germination study, project a highly significant

Table 1A. Relative percentage germination of cotton seeds.

Treatments	Day					
	2	3	4	5	6	7
Prometryne	52.38	40.63	52.94	73.53	76.47	76.47
Cotoron	28.57	34.38	38.24	38.24	38.24	38.24
Prom. + Cot. (1:1, w/w)	47.62	31.25	38.24	44.12	55.88	55.88

Table 1B. Respiratory oxygen uptake of cotton seeds during the 8th hour of their germination.

Treatments	Rate of respiration μl. O_2 uptake/seed/hr
Control	0.5190±0.044
Prometryne	0.7765±0.087
Cotoron	2.6850±0.059
Prom. + Cot. (1:1 w/w)	1.6275±0.095

(The herbicides were applied at a rate of 440 ppm–approx. 3kg/ha).

reduction in the per cent germination of cotoron-treated seeds. A reduction significant at $P < .05$ level was apparent in the seeds treated with the mixture. However, prometryne was not found to cause a significant reduction in germination (Table 2A).

The study of the respiratory behaviour (Table 1B) revealed that during the 8th hr the untreated seeds consumed 0.519±0.044 μl . O_2/seed/hr. Application of prometryne accelerated the uptake of oxygen to 0.7765± 0.087 μl . The highest rate of respiration recorded for cotoron-treated seeds was 2.685±0.059 μl . O_2 uptake/ seed/hr. A mixture of both the herbicides could raise the oxygen uptake to 1.6275±0.095 μl seed/hr. These results find their significance on a level mirror image to that of germination study (Table 2B).

Contrary to Gysin and Knusli [7], Sasaki and Kozlowski [8] and Sasaki *et al* [9], the present investigation encountered 23.53% inhibition in germination in the case of prometryne-treated cotton seeds. The increase in respiration due to the action of these herbicides is consistent with the earlier observations of Thompson *et al*.[2], and suggests that increase in respiration and concomitant decrease in the rate of germination may well be due to the uncoupling of oxidative phosphorylation by herbicide action. Similar phenomenon has been observed by a number of other

Table 2A. Analysis of variance of germination study.

Source of variation	SS	d.f	MS	F
Treatments	17.2038	3	05.7346	17.377*
Prometryne	00.9627	1	00.9627	02.917†
Cotoron	13.6486	1	13.6486	41.359*
Prom. + Cot. (1:1)	02.5925	1	02.5925	07.856**
Error	00.6590	20	00.3300	–
Total	23.7938	23	–	–

Table 2B. Analysis of variance of respiration study.

Source of variation	SS	d.f	MS	F
Treatments	881.58	3	293.86	09.0566*
Prometryne	127.81	1	127.81	03.939†
Cotoron	510.59	1	510.59	15.736*
Prom. + Cot. (1:1)	243.18	1	243.18	07.495**
Error	389.364	12	32.447	–
Total	1270.944	15	–	–

Level of significance: *$P < .001$;
† insignificant; and **$P < .05$.

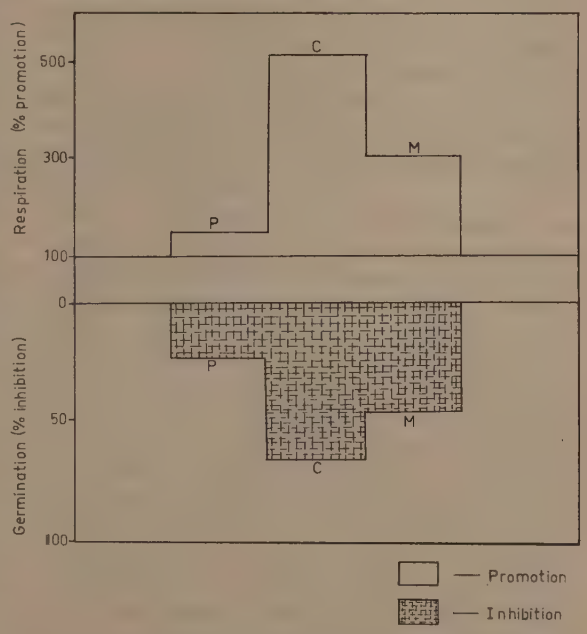

Fig 1. Relative rate of germination and respiration under the influence of prometryne, cotoron and their equal mixture [P prometryne, C cotoron, M mixture of P and C (1:1)].

workers [3,5].

Combined together, both the herbicide species do not appear to exhibit a behaviour synergistic or other wise.

A negative correlation is discernible in the two parameters studied (Fig. 1) where a decrease in the percentage germination seems inversely associated with an increase in the uptake of oxygen.

In the present investigation, oxygen uptake by delinted seeds during the 8th hour of their germination, qualifies as a good measure for a rapid evaluation of likely effects of a herbicide on the rate of germination.

It is evident that cotoron severely effected the physiology of the cotton seeds, which renders this herbicide a rather less attractive preemergence weed control measure for M-100 cotton fields. It is suggested that its application should be confined to a very limited scale as far as *Gossypium hirsutum* L.cv. M-100 is concerned. Further, a thorough probe is urged into the responses of our crops, including their varieties, to generally recommended herbicides to select and apply the appropriate herbicide or herbicide system.

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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLIII. *Ligusticum elatum* Clarke (Shangatay) Seed Oil

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A study on the essential oil obtained from the fresh mature seed of the indigenous *Ligusticum elatum* has been conducted with respect to its physicochemical characteristics and chemical composition. The oil with a yield of 0.21% is constituted of santene (0.18%), α-pinene (10.85%), camphene (3.76%), sabinene (6.33%), β-pinene (4.25%), myrcene (3.94%), β-phellandrene (0.42%), α-terpinene (0.15%), *p*-cymene (0.24%), limonene (8.82%), 1,8(9)-*p*-menthadiene (0.24%), γ-terpinene (0.27%), bornyl acetate (0.36%), linalool (0.8%), anethole (0.27%), borneol (0.42%), thujyl alcohol (0.45%), carveol (0.15%), caren-*cis*-4-ol (0.15%), dihydrocarveol (0.16%), phenyl ethyl alcohol (0.60%), β-caryophyllene (0.46%), β-elemene (2.02%), β-sesquiphellandrene (8.31%), *trans*-β-farnesene (0.52%), β-bisabolene (4.69%), β-gurjunene (2.72%), α-curcumene (0.72%), γ-bisabolene (5.06%), bergamotene (0.63%), bisabolene (3.37%), α-gurjunene (0.42%), α-farnesene (0.42%), α-bulnesene (0.96%), α-humulene (0.85%), β-selinene (1.27%) and ambrettolic acid (13.38%). The presence of ambrettolic acid in such a large amount in the essential oil of an Umbelliferae species is an interesting finding.

INTRODUCTION

Ligusticum, a genus of about 60 species of the perennial herbs, has specific significance in the plant family Umbelliferae because of its medicinal importance. The plants of the genus are restricted mostly to the north temperate regions and are well-distributed in the Himalayas from 2–3000 m. Only four species of the genus namely *Ligusticum elatum, L. marginatum, L. stewartii* and *L. thomsonii* and one variety of *Ligusticum thomsonii* called *evolutior* occur mainly in the Punjab and the North West Frontier Province and Kashmir in Pakistan.

Ligusticum elatum is a wild plant and has been reported to grow in Pakistan and Afghanistan. In Pakistan it grows in Dir, Swat and the Murree Hills. Medicinally, it has been noted to be hypotensive, central nervous depressant, stimulant of respiratory system and smooth relevant. In view of its medicinal significance, the nature of the seeential oil of the species was determined to be examined. The present investigations have been carried out to study the quality and chemistry of the essential oil of the *Ligusticum elatum* with a view to exploiting the natural resources of the country for its introduction in the local materia medica at least.

MATERIALS AND METHODS

Mature seeds of *Ligusticum elatum* were hand-collected from Madyan (Swat) for the extraction of its essential oil. As usual, the essential oil was recovered from the freshly ground seed of the species by dry steam-distillation [2]. Since the yield of the oil was low, the total aqueous distillate was extracted with diethyl ether which on removal of the solvent yielded the essential oil. The general methods employed for the physicochemical evaluation of the oil have already been given in our earlier papers [2,3]. Besides these methods, time and temperature-programmed GLC coupled with mass spectrometry was used for the resolution and examination of the essential oil.

The oil was fractionated into its hydrocarbons and oxygenated components by column chromatography using silica gel as an adsorbent. Elution of the hydrocarbon fraction was carried out with n-hexane and the oxygenated components were recovered with progressively increasing proportions of diethyl ether (2–50%) in n-hexane. The hydrocarbon fraction of the oil was further resolved into individual terpenes by GLC using a stainless-steel column (3 mm x 3 m) packed with 20% carbowax on Chromosorb G, nitrogen as the carrier gas and flame ionisation detector. The temperature was maintained at 110 and 170° for the resolution of monoterpenes and sesquiterpenes respectively. Identification of the individual constituents was carried out by coinjecting the standard reference components with the hydrocarbon fraction. The fractions containing more than one oxygenated components were either rechromatographed or preparative TLC was used to obtain single compounds. Identification of the oxygenated compounds of the oil was made by GLC and IR comparison with their

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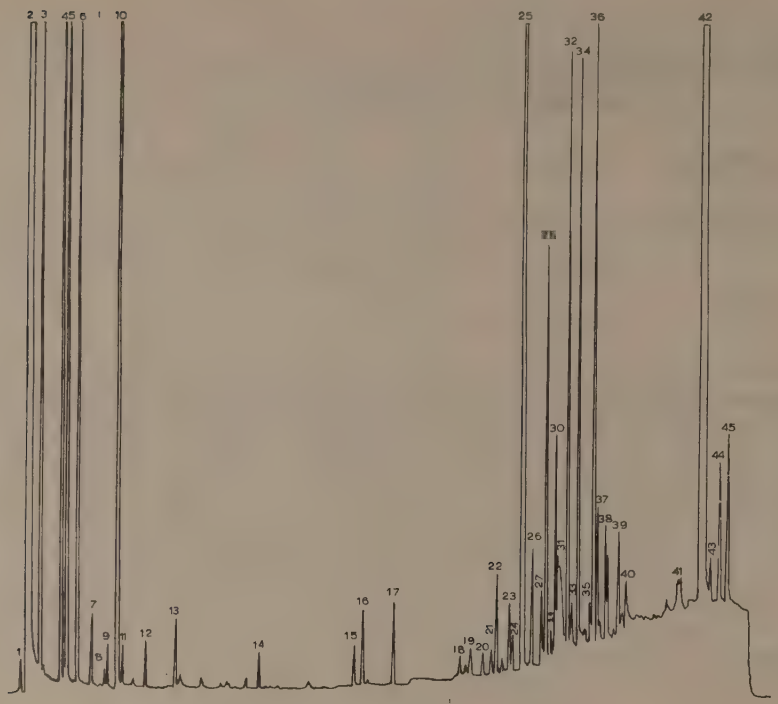

Fig. 1. Time and temperature programmed GLC of the essential oil of *Ligusticum elatum*.

standard samples. The oil was also examined as such by time and temperature-programmed GLC between 70 to 180° at the rate of 2/min.

RESULTS

The percentage yield, physicochemical properties and the chemical composition of the essential oil of the *Ligusticum elatum* are recorded in Tables 1–2. Resolution of the oil by programmed GLC is shown in Fig.1.

DISCUSSION

The essential oil obtained from the seed of *Ligusticum elatum* is reasonably sweet to smell. The oil was fractionated into terpenes and oxygenated components by column chromatography using silica gel as an adsorbent. The hydrocarbon fraction (~ 60%) of the oil consisted of monoterpenes and sesquiterpenes which were identified by GLC against their available standard samples. The major oxygenated components of the oil were separated from the column either by repeated column chromatography or by preparative TLC. Bornyl acetate, linalool, borneol, carveol, phenyl ethyl alcohol and ambrettolic acid were, separated in pur state and identified by IR comparison with their standard spectra.

Sarin [1] has reported upon the chemical composition of the essential oil obtained from the Indian variety

Table 1. Percentage yield and physico-chemical values of the essential oil of *Ligusticum elatum* seed.

Distillation time (hr)	10
Yield of oil (%)	0.21 including water-cohobation
Specific gravity	0.9125^{30}
Refractive index	1.5050^{30}
Optical rotation	$+17^{\circ}\ 10'^{12}$
Acid value	19.00
Ester value	32.00

Superscripts indicate the temperature at which these parameters were determined.

of *Ligusticum elatum* and identified α-pinene, β-pinene, salenene, Δ^3-carene and phenyl ethyl alcohol as the constituents of the oil. The present studies, on the other hand, indicate a detailed chemical composition of the Pakistani variety of *Ligusticum elatum*. Resolution of the oil by time and temperature-programmed GLC gave 45 peaks indicating 12 monoterpenes, 19 sesquiterpenes and 16 oxygenated compounds. Out of these, 37 compounds have been identified (Table 2) by comparison with their standard mass spectra.

Bornyl acetate and anethole were eluted from the column with 2% diethyl ether in n-hexane and identified against their standard IR spectra. Elution of the column with 5–10% diethyl ether in n-hexane gave a mixture of

Table 2. Percentage composition of the essential oil of *Ligusticum elatum* seed by programmed GLC.

Peak No.	Component	Percentage
1	Santene	0.18
2	α-Pinene	10.85
3	Camphene	3.76
4	Salinene	6.33
5	β-Pinene	4.25
6	Myrcene	3.94
7	β-Phellandrene	0.42
8	α-Terpinene	0.15
9	*p*-Cymene	0.24
10	Limonene	8.82
11	1,8(9)-*p*-Menthadiene	0.24
12	γ-Terpinene	0.27
13	Bornyl acetate	0.36
14	Linalool	0.18
15	Anethole	0.21
16	Borneol	0.42
17	Thujol	0.45
18	Carveol	0.15
19	Unknown	0.16
20	Caren-*cis*-4-ol	0.15
21	Dihydrocarveol	0.16
22	Phenyl ethyl alcohol	0.60
23	β-Caryophyllene	0.46
24	β-Elemene	2.02
25	β-Sesquiphellandrene	8.31
26	Unknown	1.08
27	*Trans*-β-Farnesene	0.52
28	β-Bisabolene	4.69
29	β-Farnesene	0.30
30	β-Gurjunene	0.72
31	α-Curcumene	0.72
32	γ-Bisabolene	5.06
33	Bergamotene	0.63
34	Bisabolene	3.37
35	α-Gurjunene	0.42
36	Unknown	3.53
37	α-Bulnesene	0.96
38	α-Humulene	0.85
39	β-Selinene	1.27
40	Unknown	0.81
41	-do-	0.87
42	Ambrettolic acid	13.38
43–45	Unknown	5.74

alcohols. The alcohols were separated from each other by repeated column chromatography and the resulting single compounds were identified by IR comparison with their standard samples. Phenolic fraction was eluted from the column with 20–25% diethyl ether in n-hexane. Only carveol was identifiable phenol in this fraction. Ambrettolic acid was eluted from the column with 50% diethyl ether in n-hexane. The compound was identified by IR: (3.0, 3.4, 5.9, 6.8, 7.2, 7.8, 9.0, 10.4, 13.8 nm) comparison with its standard spectra. The melting point of the acid was 20°. It was further identified by chemical method, on treatment with H_2SO_4 a lactone was obtained. However, some more work is required for the structural elucidation of this novel acid to be found in Umbelliferono species. The presence of ambrettolic acid has never been reported as constituent of the essential oils recovered from Umbelliferae. The presence of this novel compound in the essential oil of *Ligusticum elatum* is considered to be an interesting and new finding. The essential oil of the species has been analyzed in detail by programmed GLC coupled with mass spectrometry (Table 2).

The species, *Ligusticum elatum,* has been successfully cultivated in the PCSIR Laboratories, Lahore. The plant possesses valuable medicinal value. Its introduction in the local materia medica may prove useful.

Acknowledgement. We are grateful to the United States Department of Agriculture for financing this research work under a PL-480 scheme and to Mr. Abdul Waheed Sabir, our Botanist, for the identification and procurement of authentic sample for these studies.

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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLIV. *Selinum candollei*, DC (Theem) Seed Oil

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The essential oil steam-distilled from the seed of *Selinum candollei* has been characterised and studied for its physicochemical properties and chemical composition. The oil obtained in 1.5% yield is composed of 2,3-dimethyl-3-ethyl pentane (0.24%), α-thujene (0.20%), α-pinene (0.69%), phenyl methyl ketone (0.08%), santene (0.12%), 1-methyl-4-isopropenyl bensene (0.12%), salinene (1.04%), 2, 4-dimethyl hexane (0.03%), β-pinene (0.57%), myrcene (3.88%), n-undecane (1.88%), γ-terpinene (0.25%), *p*-cymene (9.00%), Δ^3-carene (5.34%), β-phellandrene (19.12%), sabinene (0.49%). 1-methyl-4-isoproyl-1, 4-cyclohexadiene (0.15%), 1,8-cineole (0.36%), 2-methyl nonane (1.65%), *o*-cymene (0.37%), n-dodecane (1.22%), verbinone (0.43%), fenchone (0.55%), cinnamaldehyde (0.28%), carvone (0.42%), anethole (0.98%), thymol (0.98%), borneol (0.73%), cuminyl alcohol (0.24%), neo-isothujyl alcohol (3.45%), methyl eugenol (0.41%), longifolene (0.49%), β-bourbonene (0.73%), alloaromandrene (0.84%), β-caryophyllene (1.39%), khusilol (0.82%), *trans*-β-farnesene (8.90%), β-elemene (1.03%), β-bisabolene (1.02%), ϵ-murrulene (0.65%), 3-*m*-tolyl-2-propanol (2.04%), α-farnesenel (2.00%) and 5βH, 7β, 10α-selina-3, 11-diene (0.18%). The essential oil of *Selinum candollei* is mainly constituted of mono- and sesquiterpenes.

INTRODUCTION

The genus *Selinum* comprises about 16 species reported chiefly from temperate regions of the northern hemisphere. Out of these, only five species, namely *Selinum candollei, S. filicifolium, S. papyraceum, S. vaginatum* and *S. wallichianum* occur in Pakistan and are perennial herbs. The plants of the genus possess a remarkable medicinal value and have long been used in dropsy, gout, epilepsy, gastrointestinal ailments and fever [1]. They are considered to be expectorant, sudorific, diuretic and emmenagogue. Some of the species of this genus have been and used as nervine sedatives and in incense. Oils of the species possess hypotensive, sedative and analgesic properties [2].

Selinum candollei grows wild from 2500 to 3500 m in the Himalayas. It has been located in the North West Frontier Province and Azad Kashmir in Pakistan. Because of the specific significance of *Selinum* genus, the medicinal importace of the indigenous *Selinum candollei* is required to be closely examined. Even though the species of *Selinum* have acquired good position in the universal materia medica, yet no work has so far appeared in literature on the chemical composition of the essential oil of these species particularly the ones growing in Pakistan. This work has, therefore, been pursued with a view to highlight the quality and chemistry of the essential oil of the Pakistani *Selinum candollei* and exploit the natural resource of the country as an item of commerce at least for its use in local materia medica.

MATERIALS AND METHODS

Mature seed of the *Selinum candollei* were collected from Kalam (Swat). The essential oil from the comminuted material was steam-distilled by using the standard procedure described in our earlier publications [3, 4]. The general methods employed for the physicochemical evaluation of the oil have already been reported [3, 4]. Because of its complex nature, the oil was studied in detail by using time and temperature-programmed GLC coupled with mass spectrometry. A Varian gas chromatograph (2100) with ionization detector was used for the resolution of the oil and identification of its various components was carried out from their mass spectra. SE-30 was used as packing material in the column and its temperature was programmed between 70–180° at the rate of 2°/min.

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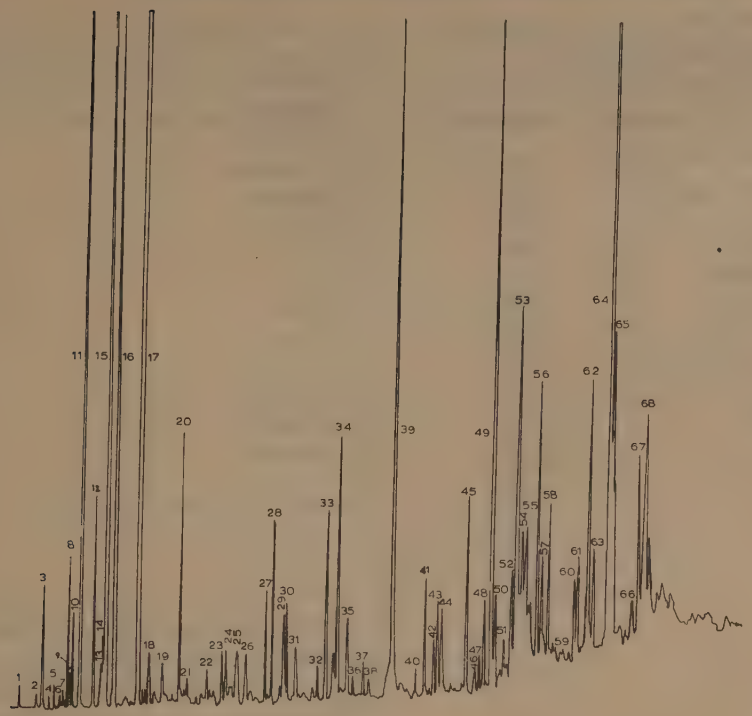

Fig. 1. Time and temperature programmed GLC of the essential oil of *Selinum candollei.*

Table 1. Percentage yield and physicochemical values of the essential oil of *Selinum candollei* seed.

Distillation time (hr)	14
Yield of the oil (%)	0.18
Specific gravity	0.9120^{25}
Refractive index	1.5160^{25}
Optical rotation	–
Acid value	8.58
Ester value	40.04

Superscripts indicate the temperature at which these parameters were determined.

RESULTS

The percentage yield, physicochemical properties and the chemical composition of the essential oil obtained from the *Selinum candollei* are reported in Tables 1–2. Resolution of the essential oil into its components by programmed GLC, is shown in Fig. 1.

DISCUSSION

The essential oil recovered from the seed of *Selinum* was column-chromatographed on silica gel using n-hexane for the elution of hydrocarbons and progressively increasing proportions of diethyl ether (2–50%) in n-hexane for the recovery of oxygenated components. The hydrocarbon fraction of the oil which consisted of monoterpenes and sesquiterpenes was further resolved into indiviual components by GLC and identified against their available standard samples. The oxygenated fractions of the oil containing more than one components were rechromatographed to obtain single compounds which were identified by GLC and IR comparison with their standard samples. However, in view of a large number of the compounds, the oil was analysed by programmed GLC coupled with mass spectrometry.

Sarin and Kapoor [2] have reported the chemical composition of the essential oil distilled from the roots of *Selinum vaginatum* and identified α-pinene, β-pinene, limonene, phellandrene, fenchone and fenchyl alcohol in the oil. The seed essential oil of *Selinum mannier* was studied by Gerasimenke and Nikonov [5] for its coumarins; osthole and 6 other coumarins were separated by chromatography. However, no work has so far been reported in literature on the essential oil of *Selinum candollei.* The present work, therefore, is the first ever of its kind particularly of the Pakistani species.

Table 2. Chemical composition of the essential oil of *Selinum candollei* seed by programmed GLC.

Peak No.	Component	Percentage
1	2,3-Dimethyl-3-ethyl pentane	0.24
2.	α-Thujene	0.20
3	α-Pinene	0.69
4	Unknown monoterpene	0.18
5	Phenyl methyl ketone	0.08
6	Santene	0.12
7	1-Methyl-4-isopropenyl benzene	0.12
8	Salinene	1.04
9	2,4-Dimethyl hexane	0.03
10	β-Pinene	0.57
11	Myrcene	3.88
12	2-Methyl nonane	1.65
13	Unknown	0.10
14	γ-Terpinene	0.25
15	*p*-Cymene	9.00
16	Δ^3-Carene	5.34
17	β-Phellandrene	19.12
18	1-Methyl-4-isopropyl-1,4-cyclohexandiene	0.15
19	1,8-Cineole	0.36
20	n-Undecane	1.88
21	*o*-Cymene	0.37
22	Fenchone	0.55
23	Sabinene	0.49
24	Verbinone	0.43
25	Unknown	0.38
26	- do -	0.57
27	- do -	0.86
28	n-Dodecane	1.22
29	Cinnamaldehyde	0.28
30	Carvone	0.42
31	Unknown	0.24
32	- do -	0.20
33	Anethole	0.98
34	Thymol	0.98
35	Borneol	0.73
36	Cuminyl alcohol	0.24
37	Unknown	0.18
38	- do -	0.24
39	Neo-isothujyl alcohol	3.45
40	Unknown	0.18
41	Methyl eugenol	0.41
42	Longifolene	0.49
43	β-Bourbonene	0.73
44	Alloaromadendrene	0.84
45	β-Caryophyllene	1.39
46	Unknown	0.04
47	- do -	0.43
48	Khusilol	0.82
49	*Trans*-β-Farnesene	8.90
50	β-Elemene	1.03
51	Unknown	0.47
52	β-Bisabolene	1.02
53	Unknown	1.63
54	∈-Murrulene	0.65
55	Unknown	0.43
56	- do -	1.80
57	- do -	0.43
58	- do -	1.23
59	- do -	0.71
60	- do -	1.22
61	- do -	0.33
62	3-*m*-Tolyl-2-propanol	2.04
63	α-Farnesene	2.00
64	Unknown	9.70
65	- do -	10.52
66	5βH-7β-10α-Selina-3,11-diene	0.18
67	Unknown	1.02
68	- do -	1.25

of terpenes (∼ 85% of the total oil). The hydrocarbon fraction of the oil contains monoterpenes and sesquiterpenes in almost equal amounts. The major monoterpene in the oil is β-phellandrene while *trans*-β-farnesene is the major sesquiterpene in the hydrocarbon fraction of the oil. The various terpenes were identified from their mass spectra.

The oxygenated fraction of the oil (∼ 15%) consists of a mixture of aldehydes, ketones, alcohols, phenols and phenolic ethers. These compounds were also identified from their mass spectra. The results of mass spectra do not show any coumarin in the oil. The last column chromatographic fraction of the oil, nevertheless, when examined under UV light, indicated a small amount of coumarins.

These studies present a detailed chemical composition of the essential oil of *Selinum candollei* growing wild in Pakistan. However, bacteriostatic/bacteriocidal activities of the oil are needed to be examined. Our successful cultivation of the plant in the PCSIR Laboratories, Lahore, will then only be materialized.

Acknowledgements. We are grateful to the United States Department of Agriculture for financing this research under a PL-480 Project and to Mr. Abdul Waheed Sabir, our Botanist for the identification and procurement of authentic material for this work.

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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLV. *Ferula assafoetida*, Linn (Herra Hing) Gum Oil

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(Received September 24, 1979)

The essential oil steam-distilled from the pure gum of *Ferula assafoetida* originating from the Baluchistan Province in Pakistan has been studied with respect to its physicochemical characteristics and chemical composition. The oil is obtained from the gum in 20.74% yield and has been found to be composed of α-pinene (14.3%), phellandrene (6.4%), secondary butyl propenyl bisulphide (51.9%), undecyl sulphonyl acetic acid (18.8%), an unidentified bisulphide (7.5%) and tarry material (1.0%). The essential oil possesses garlic-like flavour and it can be used in place of its gum which finds application in the preparation of some local dishes besides its unique medicinal value.

INTRODUCTION

Ferula is the largest genus in the Umbelliferae consisting of 140 species from the Mediterranean regions to the Central Asia. Most of these plants are reported to grow in the USSR and its adjacent regions. Pakistan is inherited only 16 species of the genus *Ferula*. The plants grow wild and are perennial herbs.

Ferula assafoetida is native to Afghanistan and Iran. The plant exudes a gum which finds wide applications as a medicine [1,2]. The gum is one of the most important species used in small quantities in most of the Indian cooking. It has since long been used in the local materia medica as stimulant, carminative, antispasmodic, expectorant, laxative, diuretic, anthelmintic, aphrodisiac, emmenagogue and vision improver. It is employed as a flavouring for sauces and other foods and for the cure of hypochondriasis. The oil of the gum has been found effective against *Staphylococcus aureus*, *Streptococcus foecatis* and *Sarcina lutea* [3].

The present investigations deal with the essential oil of the *Ferula assafoetida* gum to study its quality and chemical composition in view of exploiting the indigenous raw material of the country.

MATERIALS AND METHODS

Ferula assafoetida gum was obtained from the Quetta market where it is sold under the name of 'Herra Hing'. The essential oil from the gum was recovered by dry steam-distillation according to the standard procedure [4] The general methods used for the analysis of the oil have been reported in our earlier work [4,5].

The oil was column-chromatographed using silica gel as an adsorbent. The hydrocarbon fraction of the oil was recovered with n-hexane which was further resolved into individual components by GLC using a stainless-steel column (3 mm x 3m), nitrogen as the carrier gas and flame ionization detector. The column was operated at 110° and 175° for the resolution and identification of monoterpenes and sesquiterpenes respectively. The oxygenated components of the oil were eluted from the column with 2–10% diethyl ether in n-hexane. Identification of these compounds was carried out by IR comparison with their standard spectra and also by chemical methods.

RESULTS

The percentage yield, physicochemical values and chemical composition of the essential oil of *Ferula assafoetida* gum are recorded in Tables 1 – 2.

Table 1. Percentage yield and physicochemical values of the essential oil of *Furula assafoetida* gum.

Distillation time (hr)	14
Yield of the oil (%)	20.74
Specific gravity	0.9936^{33}
Refractive index	1.5240^{33}
Optical rotation	$-7^{\circ}\ 11^{33}$
Acid value	2.33
Ester value	31.49
Ester value after acetylation	16.94

Superscripts indicate the temperature at which these parameters were determined.

Table 2. Percentage composition of the essential oil of *Ferula assafoetida* gum.

Composition	Percentage
α-Pinene	14.3
Phellandrene	6.4
Secondary butyl propenyl bisulphide	51.9
Undecyl sulphonyl acetic acid	18.8
Unknown bisulphide	7.5
Tarry material	1.0
Unrecovered material	0.1

DISCUSSION

The characteristic strong odour of *Ferula assafoetida* gum is essentially due to the volatile oil present in it and, therefore, the quality of the gum may be expected to depend largely upon the content and composition of the volatile constituents. The gum of *Ferula assafoetida* generally available in the market is compounded one which is prepared by mixing the pure gum with either the resinous matter of other species or some additives just to increase the weight. The percentage yield of the essential oil in the pure gum was found to be about 25 times the amount of essential oil in the compounded gum. A comparison of the physicochemical constants of the two oils, however, shows that the essential oils from the two samples do not differ much.

The hydrocarbon fraction of the oil gave one major and one minor peak in the chromatogram. The major peak was identified as α-pinene and the minor one as phellandrene. No sesquiterpene was detected in the fraction under the conditions described earlier.

The oil was chiefly composed of sulphur-bearing compounds. The column when eluted with 2% diethyl ether in n-hexane gave a single compound amounting to 51.9% of the total oil. The compound was identified as secondary butyl propenyl disulphide by its IR:s (3.4, 6.3, 7.0, 8.3, 10.8 nm), m(7.3, 8.7, 13.2 nm), w(7.5, 7.7, 9.9, 10.1, 11.2, 14.3 nm). This compound has already been reported to be the major constituent of the essential oil of *Ferula assafoetida* gum.

Elution of the column with 3% diethyl ether in n-hexane gave another single compound by TLC. It was identified as undecyl sulphonyl acetic acid by IR:s (5.9 7.0, 7.6, 7.9, 8.7, 8.9, 11.0 nm), m(2.9, 6.8, 7.2, 12.2, 14.4 nm), w(9.3, 9.8, 13.1, 13.8 nm) comparison with the standard spectrum of the compound.

The column was then eluted with 10% diethyl ether in n-hexane which gave a disulphide type of compound by IR and chemical test. Identification of the compound has yet to be carried out. The column was finally washed with 100% diethyl ether but the fraction thus obtained consisted of tarry material.

The present study indicates that the essential oil from the gum of *Ferula assafoetida* constitutes almost all the components which has already been reported [1].

Acknowledgements. We are grateful to the United States Department of Agriculture for financing this research work under PL-480 scheme and to Mr. Abdul Waheed Sabir, our Botanist, for the identification and procurement of authentic material for this work.

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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLVI. *Stewartiella baluchistanica*, E. Nasir, Oil of the Whole Plant

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The essential oil steam-distilled from the whole plant of *Stewartiella baluchistanica* has been characterised and studied with respect to its physicochemical properties and chemical composition. The oil obtained in 0.15% yield consisted of : methyl benzene (1.87%), ethyl benzene (0.09%), xylene (0.13%), pentyl benzene (0.09%), 2, 3-dimethyl-3-ethyl pentane (0.52%), linalyl acetate (traces), methyl ethyl benzene (traces), 4-Tr-butyl cyclohexane (traces), *trans*-sabinone hydrate (0.07%), 2-methyl-4-hexyl hexanone (0.04%), isobutyl benzene (0.09%), phenyl methyl ketone (0.29%), *m*-menth-1, 8(9)-diene-5-ol (0.08%), cumene (0.94%), n-decane (1.30%), methyl benzaldehyde (0.15%), 1-methyl-4-isopropyl-1,3-cyclohexadiene (0.15%), 4-ethyl-1-octyn-3-ol (0.09%), pinene-oxide (0.10%), limonene-4-ol (0.17%), isocarvomenthone (1.17%), *trans*-pinocarvone (0.34%), eucarvone (1.51%), verbinone (0.55%), pulegone (5.38%), dec-3-en-2-one (0.52%), dec-4-en-2one (0.44%), thymol (0.65%), *cis-p*-menthadiene-2, 8-ol-1 (3.71%), carveol (12.34%), chavicol (0.84%), β-terpinene-3,4-oxide (0.14%), 2-methyl terephthaldehyde (2.30%), sabinol (15.83%), trimethyl benzaldehyde (0.14%), γ-muurolene (7.23%), isolongifolene (0.10%), 1-phenyl-4-methyl-octane (0.19%), γ-cadinene (0.79%), α-cadinene (0.14%), β-bisabolene (0.55%), β-elemene (1.16%), β-selinene (1.08%), β-caryophyllene (0.03%), humulene (0.28%), pinocarveol (2.76%), methyl chavicol (3.49%) and some unidentified sesquiterpenes and phenolic components. The essential oil of the *Stewartiella baluchistanica* is unique among umbellifer because none of the members of the family has so far been reported to contain such a large number of components. One hundred and nine compounds have been detected in the oil and we have succeeded in identifying about fifty of these compounds. It will be quite interesting to study the physiological effect of the oil so as to introduce the species in the local materia medica.

INTRODUCTION

The genus *Stewartiella* is named in honour of Dr. R.R. Stewart, a renowned U.S. botanist who has studied the flora of the Indo-Pakistan subcontinent. It includes only one species which is known as *Stewartiella baluchistanica*. The plant is a perennial herb and is native to Pakistan where it grows wild at Maslakh and Kachh in the Baluchistan province. No medicinal value of the species is known so far. The plant is mainly grazed by animals.

The present studies have been carried out with a view to determining the quality and chemical composition of the essential oil of the species so that the natural resources of the country may be exploited. These are the first ever studies of this kind which provide a detailed analaysis of the essential oil of *Stewartiella baluchistanica*.

MATERIALS AND METHODS

Green plants of *Stewartiella baluchistanica* were collected from Kachh (Baluchistan). The essential oil from the crushed material was recovered by dry steam-distillation according to the method described earlier [1]. The general methods used for the analysis of the oil have been reported earlier [1, 2]. Besides these methods, time and temperature-controlled gas-liquid chromatography coupled with mass spectrometry was used for the resolution of the oil and identification of the various components.

RESULTS

The percentage yield, physicochemical properties and the chemical composition of the essential oil of *Stewartiella baluchistanica* green plants have been recorded in Tables 1–2. Fig. 1 shows the resolution of the essential oil of the species.

DISCUSSION

The essential oil recovered from the whole plant of *Stewartiella baluchistanica* is quite pleasant to smell. No work has so far been appeared in literature upon the nature and chemistry of the essential oil of this species. The present studies, therefore, provide entirely new information about the physicochemical investigations on the essential oil obtained from the indigenous species of *Stewartiella*. A

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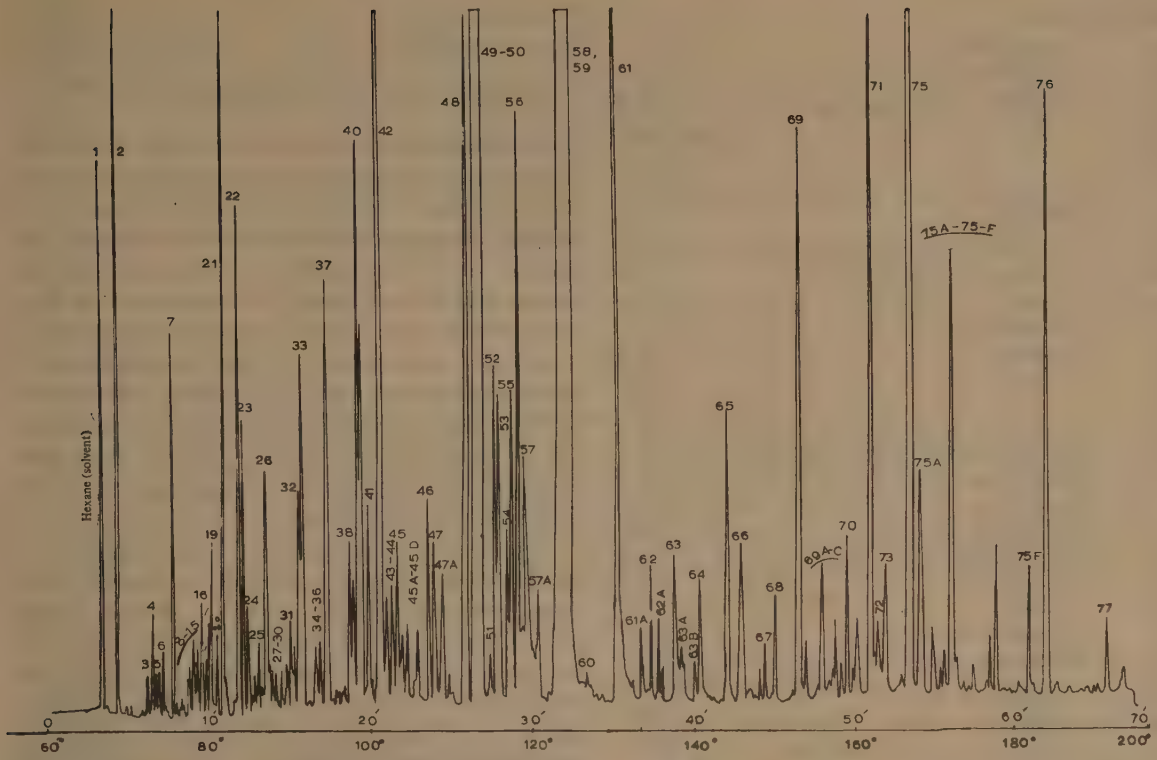

Fig. 1. Time and temperature-programmed GLC of the essential oil of *Stewartiella baluchistanica*, SE-30, 0.25 mm, $68^{0}.2^{0}$/min, 200^{0}

Table 1. Percentage yield and physicochemical values of the essential oil of *Stewartiella baluchistanica* green plants.

Distillation time (hr)	10
Yield of the oil (%)	0.15
Specific gravity	0.8910^{22}
Refractive index	1.5130^{22}
Optical rotation	$+10^{0}\ 30^{22}$
Acid value	19.21
Ester value	8.25

Superscripts indicate the temperature at which these parameters were determined.

Table 2. Chemical composition of the essential oil of *Stewartiella baluchistanica* green plant by programmed GLC.

Peak No.	Component	Percentage
1	Hexane (solvent)	1.45
2	Methyl benzene	1.87
3	Ethyl benzene	0.90
4	Xylene	0.13
5	Unknown	0.0
5A	-do-	0.06
6	Pentyl benzene	0.09
7	2, 3-Dimethyl-3-ethyl pentene	0.52
8	Linalyl acetate	Trace
9	Methyl ethyl benzene	Traces
10	4-Tr-Butyl cyclohexane	Traces
11	Unknown	0.10
12	*Trans*-Sabinene hydrate	0.07
13	Unknown	0.10
14	2-Methyl-4-hexyl hexanone	0.04
15	Isobutyl benzene	0.09
16	Phenyl methyl ketone	0.29
17	Unknown	0.07
18	*m*-Mentha-1, 8(9)-diene-5-ol	0.08
19	Unknown	0.45
20	-do-	0.10
21	Cumene	0.94
22	*n*-Decane	1.36
23	Unknown	0.58
24	Methyl benzaldehyde	0.15
25A	No mass spectra recorded	0.10
25	Unknown	0.09
26	-do-	0.49
27	Unknown	0.12
28	I-Methyl-4-isopropyl-1, 3-cyclohexadiene	0.10
29	4-Ethyl-l-octyn-3-ol	0.09
30	α-Pinene oxide	0.10
31	Unknown	0.24
31A	-do-	0.09
32	-do-	0.28
33	-do-	0.95
34, 35	Limonen-4-ol	0.17
36	Unknown	0.19
37	Isocarvomenthone	1.17
38	Unknown	0.45
39	*Trans*-Pinocarvone	0.34
40	Eucarvone	1.15
41	Verbinene	0.55
42	Pulegone	5.38
43	Unknown	0.06

(Table 2 continued)

44	Dec-3-en-2-one	0.52
45	Dec-4-en-2-one	0.44
45A	Unknown	0.21
45B	-do-	0.14
45C	-do-	0.22
45D	-do-	0.19
46	Thymol	0.83
47	Unknown	0.65
47A	-do-	0.72
47B	-do-	0.08
48,49	*Cis-p*-Menthadiene-2-8-ol-1	Traces
50	Carveol	12.35
51	β-Terpinen-3,4-oxide	0.14
52	Unknown	0.91
53	-do-	0.82
54	-do-	0.48
55	-do-	0.84
56	2-Methyl terephthaldehyde	2.30
57A	Unknown	0.17
57	-do-	1.34
58, 59	Sabinol	15.38
60	Trimethyl benzaldehyde	0.14
61	γ-Muurolene	7.23
61A	Isolongifolene	0.10
61B	Unknown	0.31
62	1-Phenyl-4-methyl-octane	0.19
62A	Unknown	0.11
62A	-do-	0.04
63	γ-Cadinene	0.79
63A	α-Cadinene	0.14
63B	Unknown	0.12
64	β-Bisabolene	0.55
65	β-Elemene	1.16
66	β-Selinene	1.08
66A	Unknown	Traces
67	β-Caryophyllene	0.03
68	Humulene	0.28
69	Unknown	4.60
69A	-do-	0.15
69B	-do-	0.34
69C	-do-	0.09
69D	-do-	0.04
70	-do-	0.43
70A	-do-	0.10
70B	-do-	0.03
71	Pinocarveol	2.76
72	Unknown	0.20
73	-do-	0.38
74	-do-	0.03
75	Methyl chavicol	3.49
75A to 75I	Unknown	5.41
76	-do-	4.00
77	-do-	0.31

special significance is attached to the present work firstly because the hydrocarbon fraction of the oil is chiefly composed of aromatic compounds which is in contrary to the chemistry of the hydrocarbon fractions of the essential oils obtained from the Umbelliferae species and secondly because such a large number of components have been detected in the essential oil of any member of the family.

It is quite an interesting fact that the essential oil obtained from the whole plant of *Stewartiella baluchistanica* has been resolved into one hundred and nine components. Out of these we were able to identify about fifty compounds by comparison with the standard mass spectra of the individual constituents.

The essential oil has been found to be a mixture of monoterpenes, aromatic hydrocarbons, sesquiterpenes, esters, aldehydes, ketones, alcohols, phenols, phenol ethers, terpene oxides and possibly some acidic compounds and coumarins. The hydrocarbon fraction of the oil consisted mainly of aromatic compounds. These compounds have not been identified in most of the essential oils of the umbelliferous plants. It is essential to carry out further studies upon the physiological nature of the essential oil.

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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part XLVII. *Angelica archangelica*, Linn. var. Himalaica (Clarke), E. Nasir, Seed Oil

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A study upon the physicochemical characteristics and chemical composition of the essential oil distilled from the *Angelica archangelica* has been carried out. The percentage yield of the oil is 0.4% and its constituents are: α-pinene (11.4%), camphene (1.9%), limonene (0.8%), α-phellandrene (0.6%), β-phellandrene (2.1%), *p*-cymene (1.4%). β-caryophyllene (2.4%), β-bisabolene (1.5%), hexyl methyl phthalate (36.0%), an unknown acid (1.6%), a mixture of coumarins (18.1%), bergaptene (19.6%) and oxypeucedanin (2.6%). The major oxygenated component of the oil namely hexyl methyl phthalate is a new find in the essential oil of the Pakistani *Angelica archangelica* seed.

INTRODUCTION

Angelica is a large genus having some 80 species which occur in the northern hemisphere. The heaviest concentration of the species of the genus is met with in Japan. So far twenty-three of the species including nine which are endemic have been reported to grow in this country. Sixteen of the species containing two that are endemic, occur in the USSR while eight species are distributed in Europe. Only two species and one variety namely *Angelica archangelica*, var. *Himalaica, Angelica glauca* and *Archangelica oreodum* have been reported to grow in Pakistan. The plants are perennial herbs. The *Archangelica* spp is sometimes kept separate from the species *Angelica* and sometimes included in them.

Angelica archangelica is native to Europe and the Eastern United States where it grows wild. The plant is also cultivated in Germany and France for its roots and seeds for the extraction of essential oil to be used in various food products. The roots and the seeds of the plant are also used in medicine for various ailments. Prolonged distillation of seeds and roots yields an oil of superior quality. This oil contains lactones which are the most important and characteristic constituents so far as odour and flavour are concerned.

The present investigations have been carried out to study the quality and chemistry of the essential oil obtained from the indigenous *Angelica archangelica* seeds.

MATERIALS AND METHODS

The seeds of *Angelica archangelica* were collected from Naran in the Kaghan valley. The seeds were crushed and steam-distilled as usual [1]. The oil was recovered by extraction of the aqueous distillate with diethyl ether. The general methods used for the analysis of the essential oils have already been communicated [1, 2]. Besides these methods, a Beckman DB spectrophotometer was used to record the UV spectra of coumarins.

The essential oil was resolved into hydrocarbon fraction and oxygenated components by column chromatography using activated silica gel as an adsorbent. The hydrocarbon fraction of the oil was eluted with n-hexane and the oxygenated components with increasing proportions of diethyl ether (1–10%) in n-hexane. The lactones present in the oil were, however, eluted from the colum with pure diethyl ether. Ten fractions were collected and those with identical behaviour by TLC and IR were mixed thus giving rise to four final fractions which were further resolved and their constituents identified by GLC and IR.

RESULTS

The percentage composition of the essential oil obtained from the seeds of *Angelica archangelica* is recorded in Table 1.

DISCUSSION

The hydrocarbon fraction of the essential oil of the *Angelica archangelica* when resolved by GLC gave six monoterpenes and two sesquiterpenes which were identi-

Table 1. Chemical composition of the essential oil of *Angelica archangelica* seeds.

Eluent	Component	Percentage
n-Hexane	Hydrocarbons*	22.1
	α-Pinene	11.4
	Camphene	1.9
	Limonene	0.8
	α-Phellandrene	0.6
	β-Phellandrene	2.1
	c-Cymene	1.4
	β-Caryophyllene	2.4
	β-Bisabolene	1.5
2% Diethyl ehter in *n*-hexane	Hexyl methyl phthalate	36.0
10% 〃	An unknown acidacid	1.6
100% 〃	Mixture of coumarins	18.1
	Bergaptene	19.6
	Oxypeucedanin	2.6

*: Resolved and estimated by GLC.

fied by coinjecting with their standard samples.

Bakina and Senchenko [3] have analyzed the essential oil of the fruit of *Angelica archangelica* by GLC and reported α-pinene, β-pinene, myrcene, β-phellandrene, α-ter pinene, *p*-cymene, β-bisabolene, β-elemene, γ-cadinene, humulene and α-bisabolol as the constituents of the oil. We have on the other hand, identified α-pinene, camphene, limonene α- and β-phellandrene, *p*-cymene, β-caryophyllene and β-bisabolene in the hydrocarbon fraction of the essential oil of the indigenous *Angelica archangelica.*

The major oxygenated component in the oil under discussion was found to be hexyl methyl phthalate. Identification of this compound depended on IR analysis followed by NMR results. However, some more chemical work is needed for the confirmation of this novel compound to be present in the essential oil of an Umbelliferae species.

Bakina and Senchenko [3] have reported α-bisabolol in the essential oil of *Angelica archangelica.* We could not detect this sesquiterpenic alcohol in our oil. Instead an acid was extracted from the Pakistani species which is still to be identified. The presence of hydroxy myristic acid has also been reported by the earlier worker [3, 4] in the essential oil of *Angelica archangelica.*

The essential oil from the Pakistani species also contained a considerable amount of coumarins which contributed mainly to the characteristic smell of the oil. We were able to separate and identify only two coumarins, namely, bergaptene and oxypeucedanin. The presence of coumarins in the essential oil of *Angelica archangelica* has already been reported by the earlier workers [3, 4].

In the light of the present studies it can be concluded that the major oxygenated component of the oil is a new finding in the essential oil of the Pakistani *Angelica archangelica* seeds.

The commercial importance of the species can only be clear after studying the bacteriostatic/bacteriocidal activities of the oil. However, the medicinal values of the oil as reported earlier are quite promising.

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FATTY ACID COMPOSITION OF *MELIA AZEDARACH* SEED OIL (BAKAIN)

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Fatty acid composition of the Bakain seed has been investigated. It was found to contain palmitic (6.93%), stearic (2.98%), oleic (8.2%) and linoleic (82.05%) acids. Possibility of its commercial exploitation has been discussed.

INTRODUCTION

Melia azedarach Linn, belongs to Meliaceae family and is commonly known as 'bakain' or 'drek.' The bakain tree is commonly grown in the Indo-Pakistan subcontinent as an ornamental shade tree. It also grows wild at altitudes between 2000–3000 ft above sea level. The various parts of the tree are reputed to have the same therapeutic values as those of nim tree [1]. In the Punjab its seeds are prescribed in reheumatism. The bakain fruit has been analysed and it is known that the seed contains fixed oil (30%) [1].

Konovalov *et al.* [2] also confirm the domination of linoleic acid in the oil of *M. azedarach,* however, it has been claimed that the longer fruits have higher percentage of unsaturation whereas the smaller ones have lower percentage of unsaturated fatty acids. Eckey [3] reviews the literature up to about 1950 in which it has been shown that the China berry *(M. azedarach)* contains mostly linoleic and oleic acids. It is also mentioned that the oil has been used in India for soap making. More recently Kaplan [4] reports the chemical composition of the African variety of *M. azedarach* in which GLC of the fatty acid ester has been reported. It is also known that the fruit of *M. azedarach* is poisonous [4]. This work deals with the detailed analysis of the oil from seeds of the fruit of *Melia azedarach,* harvested in Pakistan. The fruit used in this paper was completely mature fruits of the species found predominatly in the Punjab.

EXPERIMENTAL

The bakain seeds were ground in pestle and mortar and extracted in a Soxhlet extractor with hexane. Removal of the solvent and drying over anhydrous sodium sulphate gave a pale yellow oil (30%). With an iodine value 136.50. The physicochemical properties of the oil determined by standard methods [5, 6] are: Refrective index 1.428, saponification value 269.20, iodine value 136.50, peroxide value 8.80, specific grevity 0.89, and acid value 1.20.

The methyl esters [7] of the fatty acids were prepared directly from the oil with methanol, acetyl chloride and benzene. GLC of the esters after drying over sodium sulphate in petroleum ether was done on a Pye Unicam 104 Series using a glass column 1.5 mx4 mm packed with 20% DEGS on diatomite (80–100) mesh. Column temperature was maintained at 180° and nitrogen gas was used as the carrier gas. Detection was made by flame ionisation detector.

DISCUSSION

The fatty acid composition of the bakain seed oil

Table 1. Some linoleic acid-rich oil seeds.

Taxonomic name	Local name	Linoleic acid contents (%)	Ref
Oilseeds of Importance			
Helianthus annuus	Sunflower	44.2–75.4	8
Carthamus tinctorius	Safflower	72.9–79.0	3
Nicotina tabacum	Tambakoo	60–77.3	15
Papaver somniferum	Khashkhash	62.2–73.0	8
Oilseeds of Minor Importance			
Salvia spinosa	Kanucha	89.5	13
Ocimum pilosum	Tukhm-i-Rehan	89.8	13
Morus alab	Shahtut	84.06	14

reveals that it contains linoleic acid as a major component alongwith palmitic (C-16, 6.93%), stearic (C-18, 2-98%), oleic (C-18:1, 8.02%), and linoleic (C-18:2 82.05%) acids. Some previously reported linoleic acid-rich seed oils [6, 8] are given in Table 1.

It can be seen that the bakain seed oil falls amongst the top-three-highly-rich linoleic acid containing oil seeds. Nutritive and medicinal values of linoleic acid in vegetable oils have been discussed elsewhere [9–12].

However, there are a number of factors which may affect the commercial exploitation of the bakain seeds as a source of linoleic acids. Firstly the difficulty of collecting seeds which can be overcome by the cooperation of forest department of the provinces as an average bakain tree gives about a maund of dry fruit during season (March–April). Secondly the low percentage of seed in the whole fruit (6.80%). It has been found that linoleic acid separates essily from the bakain seed oil thus the seeds can be a good source of linoleic acid.

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COLOURED GLAZES BASED ON SURFACE CLAY

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Surface clay (red clay) has been used as a base for various coloured glazes such as yellow, brown, maroon, black and light blue, maturing between 1000^{o}–1100^{o}. Iron content of the clay has been utilized for developing these colours. Additional Fe_2O_3 and MnO_2 have been added for effecting desirable changes in the shade of some glazes.

INTRODUCTION

Surface clay is the cheapest raw-material and this has been the main reason for carrying out this study. We have tried to develop coloured raw glazes which may be used for artware and can be applied on the earthenware body-composition usually used in local pottery industry at Gujrat and Gujranwala. Surface clay might have been used in isolated cases as a glaze component but no systematic study has been reported for the development of coloured glazes based on surface clay. Effect of iron content on the colour of ceramic products has been studied by Macvay and Parmellee [1]. They have pointed out the possibility of producing red, tan and brown colours in engobes and other ceramics products. Hostetter and Roberts [2] have studied the effect of glass composition on the colour imparted by iron oxide. Konnision [3] has studied the effect of iron-content on the colour of clay.

EXPERIMENTAL

Red clay, K.D. pottery clay, feldspar, quartz, talc, laterite and soda-lime glass (ordinary bottle glass) were the raw-materials used. The chemical analysis is reported in Table 1.

In addition to the technical grade zinc oxide, white lead, manganese dioxide and calcuim fluoride were also used.

Procedure

Washed surface clay, quartz and feldspar, were ground to 100 mesh. Various constituents of glaze were mixed in proper proportions and the mix was ball-milled for 5–6 hr after the addition of water. The consistency of the glaze was adjusted and it was applied to test pieces by dipping or spraying in the green state as well as after biscuit firing at 900^{o}. In the case of earthenware body used by local pottery industry the glaze was applied to pieces fired at 900^{o} as well as at 1150^{o}. The compositions of these two bodies are: Gujrat body containing talc (50%), K.D. pottery clay (25%), whiting (6%), glass (14%) and quartz (5%), and Artware body containing K.D. pottery clay (60%), feldspar (18%) and quartz (22%).

Test pieces from these bodies were made by routine casting procedure.

Table 1. Chemical analysis

	Red clay	K.D. pottery clay	Quartz	Feldspar	Talc	Laterite	Soda-lime glass
I/L	6.20	13.60	0.21	0.35	7.72	9.65	0.10
SiO_2	59.60	47.10	97.13	66.90	58.10	12.28	70.00
Al_2O_3	19.28	32.98	1.39	21.28	2.52	23.57	3.30
Fe_2O_3	5.38	1.12	0.035	0.026	1.13	49.33	0.25
CaO	3.74	1.40	0.88	0.12	0.73	0.24	8.80
MgO	2.70	1.04	0.35	0.09	29.15	1.23	2.70
Na_2O	2.07	1.50	–	8.67	0.42	–	14.10
TiO_2	0.3	0.76	–	–	–	3.99	–
K_2O	0.63	0.48	–	2.25	0.20	–	0.58

Table 2. Yellow glazes

Composition No.	Clay (%)	Feldspar (%)	Quartz (%)	ZnO (%)	White lead (%)	Glass (%)	Talc (%)	Colour (%)
1	90–80			10–20				Yellow
2	70–50			30–50				Dirty cream
3	50			5	45			Brownish yellow
4	50			10	40			Yellow
5	50			15	35			"
6	50			20	30			"
7	45.5		45.5		9			"
8	43.5		43.5		13			Good yellow
9	41.7		41.7		16.6			"
10	45.5				9.0	45.5		Reddish up to 1000°
11	43.5				13.0	43.5		& yellow above 1050°
12	41.7				16.6	41.7		"
13	45.5	45.5			9.0			Reddish yellow
14	43.5	43.5			13.0			"
15	41.7	41.7			16.6			"
16	40.0	40.0			20.0			"
17	38.5	38.5			23.0			"
18	45.5				45.5		9.0	Bronish yellow
19	43.5				43.5		13.0	"
20	41.7				41.7		16.6	"
21	40.0				40.0		20.0	"
22	38.5				38.5		23.0	"
23	31.3		12.4		31.3	18.8	6.2	Bright yellow
24	30.3		12.1		30.3	18.2	9.1	"
25	29.4		11.75		29.4	17.7	11.75	"

DISCUSSION

Variations in the colour of ceramic products containing only iron oxide as a colouring agent are due to the fact that iron can exist in the Fe^{++} and Fe^{+++} form and the ratio of Fe^{++} to Fe^{+++} is the controlling factor as regards the shade of the colour [1–3]. This ratio can be controlled or modified by factors such as composition of the glaze, firing temperature and the atmosphere of the furnace. In glazes (glasses) Fe^{3+} behaves as a network former and Fe^{2+} as a network modifier [3].

Yellow Glazes. Hostetter *et al.* [2] have produced a lemon yellow shade in a soda-lime-silica glass composition by controlling the Fe^{2+}/Fe^{3+} ratio. In the present study for developing a yellow glaze the presence of 10–20% ZnO is necessary to keep the Fe^{2+}/Fe^{3+} ratio within such values where the shade is yellow. In the absence of ZnO the white lead component of the glaze helps Fe^{3+} to act as a network former and the glaze turns brown. Even in the presnece of ZnO, if the amount of white lead is more than 30% the colour changes to brown. ZnO can be replaced by talc and the same yellow shade can be obtained.

Compositions giving yellow shade have been reported in Table 2. It is clear from the results that a glaze with good yellow shade can be obtained by adding ZnO or quartz to red clay. Other additions are made to have desirable physical properties in the glaze. When 10–20% of ZnO is added to red clay, the yellow colour is developed but the glaze remains matt even above 1100°. Substitution of 35–40% of clay in the above composition with white lead gives good yellow glaze maturing above 1000°. Addition of quartz to red clay in the presence of fluxes, also produces yellow shade. When 1–20% of white lead is added to a 1:1 mix of clay and quartz, a good yellow-coloured glaze is obtined which matures above 1050°.

Physical properties like texture and lustre are improved by increasing the amount of white lead to 30% and replacing 12% clay and 10% quartz with talc and glass. Small additions of CaF_2 increase the brightness of the glaze.

Brown Glazes. For brown coloured glazes the presence of white lead is necessary because it helps Fe^{3+} to enter into four-fold coordination just like silicon atoms and the

Table 3. Brown glazes

Composition No.	Clay	White lead	Glass	Laterite	Quartz	Temp. (°C)	Colour
1	60	40				950	Reddish brown
2	50	50				900	Brown
3	38.5	38.5	19.2	3.8		1050	Brown
4	37.6	37.6	18.8	6.0	–	1050	Brown
5	37.0	37.0	18.5	7.5	–	1050	Reddish brown
6	36.5	36.5	18.25	8.75	–	1050	Coffee brown
7	35.7	35.7	17.9	10.7	–	1050	Coffee brown
8	31.25	31.25	18.7	6.3	12.5	1100	Choccolate brown
9	30.3	30.3	18.2	6.05	15.15	1100	Brown
10	29.4	29.4	17.65	5.9	17.65	1120	Reddish Brown

Table 4. Black glazes

Composition No.	Clay	Glass	White lead	MnO	Temp. (°C)	Colour
1	42.4	12.7	42.4	2.5	1040	Brown-reddish black
2	41.3	12.4	41.3	5.0	"	Black
3	40.7	16.3	40.7	2.4	"	Brownish-reddish black
4	39.7	15.9	39.7	4.7	"	Black
5	39.1	19.5	39.1	2.3	"	Brownish black
6	38.2	19.1	38.2	4.5	"	Black

Table 5. Fritted glazes

S.No.	Red clay	White lead	Quartz	Glass	Zinc oxide	Talc	Laterite	MnO_2	Colour
1	50.0	30.0	–	–	20.0	–	–	–	Yellow
2	43.5	9.0	45.5	–	–	–	–	–	"
3	41.7	16.6	41.7	–	–	–	–	–	"
4	30.3	30.3	12.1	18.2	–	9.1	–	–	"
5	37.0	37.0	–	18.5	–	–	7.5	–	Brown
6	31.25	31.25	12.5	18.7	–	–	6.3	–	"
7	39.7	39.7	–	15.9	–	–	–	4.7	Black

colour of Fe^{+++} in such environment is brown. When the iron is completely in the four-fold coordination we can get very uniform brown glazes. The amount of white lead sufficient to help iron enter the four-fold coordination completely, is about 30%. In the case of lower amount the glaze remains brick red.

Choccolate-coloured glaze is obtained by the addition of white lead to red clay. For a good choccolate colour, the amount of white lead should be more than 40%. Below 40% the colour remains brick-red. Choccolate-coloured glaze matures at 1050°. Addition of glass up to 20% in a 1:1 mixture of clay and white lead produces

reddish brown colour but, when additional Fe_2O_3 is introduced in the form of laterite, the colour changes, first to coffee-brown and then to blackish brown. Compositions in Table 3 have very good coffee-brown shade with metallic lustre. The amount of laterite necessary to produce coffee-brown is 7–10%. More than 10% laterite imparts a balckish tinge. When about 12% quartz is added to a composition containing 6% laterite at the expense of clay and white lead, we get a choccolate-brown colour. Increasing additions of quartz lighten the colour and with 18% quartz in the glass we get a light brown colour with a metallic lustre.

Black Glazes. Composition presented in Table 4 show that a 1:1 mixture of red clay and white lead gives a choccolate colour. Addition of soda-lime glass up to 20% imparts desirable properties of texture and gloss alongwith metallic lustre. Metallic lustre and gloss increase with, increasing glass content. These glazes mature at about 1050°.

Fritted Glazes. Some of the glaze compositions given in Tables 2–4 were fritted to see their behaviour as regards the colour and other properties. The frit compositions are presented in Table 5. It has been found that the fritted composition when applied with an addition of 5% K.D. clay as mill addition give satisfactory results. The colour remains the same but there is a slight change in the shade of the colour.

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